#6,673,533
#6,258,870

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☐ 1. Document ID: US 6485703 B1

L3: Entry 1 of 6 File: USPT Nov 26, 2002

US-PAT-NO: 6485703
DOCUMENT-IDENTIFIER: US 6485703 B1

TITLE: Compositions and methods for analyte detection

DATE-ISSUED: November 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cote ; Gerard L.	College Station	TX		
Pishko; Michael V.	College Station	TX		
Sirkar; Kaushik	College Station	TX		
Russell; Ryan	College Station	TX		
Anderson; Richard Rox	Lexington	MA		

US-CL-CURRENT: 424/9.1; 424/9.6, 424/9.8

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Drawi De

☐ 2. Document ID: US 5618265 A

L3: Entry 2 of 6 File: USPT Apr 8, 1997

US-PAT-NO: 5618265
DOCUMENT-IDENTIFIER: US 5618265 A

TITLE: Iontophoretic delivery device with single lamina electrode

DATE-ISSUED: April 8, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Myers; Robert M.	Stanford	CA		
Landrau; Felix A.	San Jose	CA		

US-CL-CURRENT: 604/20; 607/115

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Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 3. Document ID: US 5543098 A

L3: Entry 3 of 6 File: USPT Aug 6, 1996

US-PAT-NO: 5543098
DOCUMENT-IDENTIFIER: US 5543098 A

TITLE: Iontophoretic delivery device and method of making same

DATE-ISSUED: August 6, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Myers; Robert M.	Stanford	CA		
Landrau; Felix A.	San Jose	CA		

US-CL-CURRENT: 264/104; 264/105, 264/126, 604/20

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 4. Document ID: US 5525356 A

L3: Entry 4 of 6 File: USPT Jun 11, 1996

US-PAT-NO: 5525356
DOCUMENT-IDENTIFIER: US 5525356 A

TITLE: Amphoteric N-substituted acrylamide hydrogel and method

DATE-ISSUED: June 11, 1996

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Jevne; Allan H.	Anoka	MN		
Holmblad; Carolann	Cambridge	MN		
Phipps; Joseph B.	Plymouth	MN		
Howland; Warren W.	Champlin	MN		

US-CL-CURRENT: 424/484; 424/486, 514/944

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 5. Document ID: US 5405317 A

L3: Entry 5 of 6 File: USPT Apr 11, 1995

US-PAT-NO: 5405317

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DOCUMENT-IDENTIFIER: US 5405317 A

TITLE: Iontophoretic delivery device

DATE-ISSUED: April 11, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Myers; Robert M.	Menlo Park	CA		
Stahl; Mark G.	Fremont	CA		
Landrau; Felix A.	San Jose	CA		
Gyory; J. Richard	San Jose	CA		

US-CL-CURRENT: 604/20; 607/149

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw De

☐ 6. Document ID: US 5147297 A

L3: Entry 6 of 6 File: USPT Sep 15, 1992

US-PAT-NO: 5147297
DOCUMENT-IDENTIFIER: US 5147297 A
**** See image for Certificate of Correction ****

TITLE: Iontophoretic delivery device

DATE-ISSUED: September 15, 1992

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Myers; Robert M.	Stanford	CA		
Stahl; Mark G.	Sunnyvale	CA		

US-CL-CURRENT: 604/20; 607/152

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw De

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L10: Entry 1 of 4 File: USPT Sep 11, 2001

DOCUMENT-IDENTIFIER: US 6289242 B1
TITLE: Electrotransport system with ion exchange material competitive ion capture

Brief Summary Text (6):
During the electrotransport process certain modifications or alterations of the skin may occur such as increased ionic content, hydration, dielectric breakdown, extraction of endogenous substances and electroporation. Any electrically assisted transport of species enhanced by modifications or alterations to a body surface (eg, formation of pores in the skin) are also included in the term electrotransport as used herein.

Brief Summary Text (31):
U.S. Pat. No. 4,731,049 to Parsi discloses an iontophoresis device employing a drug reservoir in which the drug to be delivered is initially bound to an ion exchange medium or an immobilized ligand affinity medium. Ions such as hydrogen (H.sup.+), sodium, potassium, hydroxyl, chloride, and sulfate ions are generated at the electrode or provided by an ion reservoir and are exchanged for the bound drug ions, thereby releasing the drug ions for delivery into the patient's body. Parsi discloses a donor electrode assembly having a hydrophilic polymer-based electrolyte reservoir and drug reservoir layers, a skin-contacting hydrogel layer, and optionally one or more semipermeable membrane layers. The ion exchange media is disclosed to be in the form of beads, powder, packed fibers, woven or knit fibers, microporous or macromolecular resin or liquid resin. Parsi employs electrodes which are electrochemically catalytic, ie, the electrodes are composed of materials (eg, carbon, graphite or metal, such as platinum group metals) which catalyze the electrochemical reaction as described above. Parsi is limited in its application to systems where drug can be bound to an ion exchange resin or medium or an immobilized ligand affinity medium, and for this reason, must possess a charge opposite that of the drug ion. U.S. Pat. No. 4,915,685 to Petelenz et al discloses a system closely related to that disclosed by Parsi.

Brief Summary Text (39):
Size exclusion, in the case of a size selective membrane, means simply that the pore size of the membrane is too small to permit specific molecules or ions to pass. Physical size or molecular weight restriction prevents or hinders the passage of species through the membrane. Utilization of size selective membranes also can create polarization as discussed above if the "excluded" species tend to have the same (+/-) charge.

Detailed Description Text (24):
The composition of the active or donor electrode assembly of the invention may include ingredients to control or alter their physical properties. Surfactants may be added to a drug reservoir to control the active agent release rate. Humectants may be added to the drug reservoir to control the evaporative loss of water. Preservatives may be added to extend the shelf life of the product. Inert fillers may be added to control the bulk density or to dilute or adjust other properties. Tackifiers may be added to enhance the adhesion of the hydrogel to the skin, electrode, or other structural components of the system. Preferably, the physical properties are adjusted so that the electrode assembly is substantially solid, that is, its consistency is such that the material does not perceptively flow.

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L16: Entry 1 of 15 File: USPT Jan 6, 2004

DOCUMENT-IDENTIFIER: US 6673533 B1
TITLE: Multi-array multi-specific electrochemiluminescence testing

Abstract Text (1):
Materials and methods are provided for producing patterned multi-array, multi-specific surfaces for use in diagnostics. The invention provides for electrochemiluminescence methods for detecting or measuring an analyte of interest. It also provides for novel electrodes for ECL assays. Materials and methods are provided for the chemical and/or physical control of conducting domains and reagent deposition for use multiply specific testing procedures.

Brief Summary Text (9):
Electrochemiluminescence ("ECL") is the phenomena whereby an electrically excited species emits a photon (see, e.g., Leland and Powell, 1990 J. Electrochem. Soc. 137 (10):3127-3131). Species from which ECL can be induced are termed ECL labels and are also referred to herein as TAGs. Commonly used ECL labels include: organometallic compounds where the metal is from, for example, the noble metals of group VIII, including Ru-containing and Os-containing organometallic compounds such as the Ru(2,2'-bipyridine).sub.3.sup.2+ moiety (also referred to as "Rubpy" or TAG1), disclosed, e.g., by Bard et al. (U.S. Pat. No. 5,238,808). "TAG1" and "Rubpy" also refer to derivatives of Ru(2,2'-bipyridine).sub.3.sup.2+. Fundamental to ECL-based detection systems is the need for an electrical potential to excite the ECL label to emit a photon. An electrical potential waveform is applied across an electrode surface, typically a metal surface, and a counterelectrode (see e.g., U.S. Pat. Nos. 5,068,088, 5,093,268, 5,061,445, 5,238,808, 5,147,806, 5,247,243, 5,296,191, 5,310,687, 5,221,605). The ECL is promoted to an excited state as a result of a series of chemical reactions triggered by the electrical energy received from the working electrode. A molecule which promotes ECL of the TAG is advantageously provided, such as oxalate or, more preferably, tripropylamine (see U.S. Pat. No. 5,310,687).

Brief Summary Text (10):
The excitation of a TAG in an ECL reaction typically involves diffusion of the TAG molecule to the surface of an electrode. Other mechanisms for the excitation of a TAG molecule by an electrode include the use of electrochemical mediators in solution (Haapakka, 1982, Anal Chim. Acta, 141:263) and the capture of beads presenting TAG molecules on an electrode (PCT published applications WO 90/05301 and WO 92/14139). Alternatively, ECL has been observed from TAG that was adsorbed directly on the surface of working electrodes (U.S. Pat. No. 5,324,457), e.g., by non-specific adsorption (Xu et al., 1994, Langmuir, 10:2409-2414), by incorporation into L-B films (Zhang et al., 1988, J. Phys. Chem., 92:5566), by incorporation into self-assembled monolayers (Obeng et al., 1991, Langmuir, 7:195), and by incorporation into thick (micrometer) films (Rubenstein et al., 1981, J. Am. Chem. Soc., 102:6641). Similarly, Xu et al. (PCT published application WO 96/06946) have observed ECL from TAG molecules intercalated into DNA strands when such strands were adsorbed onto gold electrodes by interaction with aluminum centers immobilized on a self-assembled monolayer of alkanethiolates.

Brief Summary Text (11):
Various apparatus well known to the art are available for conducting and detecting

ECL reactions. For example, Zhang et al. (U.S. Pat. No. 5,324,457) discloses exemplary electrodes for use in electrochemical cells for conducting ECL. Leventis et al. (U.S. Pat. No. 5,093,268) discloses electrochemical cells for use in conducting ECL reactions. Kamin et al. (U.S. Pat. No. 5,147,806) discloses apparatus for conducting and detecting ECL reactions, including voltage control devices. Zoski et al. (U.S. Pat. No. 5,061,445) discloses apparatus for conducting and detecting ECL reactions, including electrical potential waveform diagrams for eliciting ECL reactions, digital to analog converters, control apparatus, detection apparatus and methods for detecting current generated by an ECL reaction at the working electrode to provide feedback information to the electronic control apparatus.

Brief Summary Text (14):
To date, all commercial ECL assays are carried out on centimeter scale electrode surfaces. The centimeter scale electrodes strike a balance between the enhanced magnitude of an ECL signal resulting from larger electrodes and the desirability of decreasing the total sample volume necessary for each assay. However, even centimeter scale electrodes fail to achieve the sensitivity required for many assays. In an attempt to overcome this problem, all commercial ECL systems further enhance sensitivity by using coated magnetic beads to capture ECL analytes or reagents. The beads are then moved adjacent to a working electrode for enhanced sensitivity.

Brief Summary Text (17):
Commercial methods for conducting ECL assays also require that the assay cell, including the electrodes, must be cleaned by any one of a number of methods, including the use of dilute acids, dilute bases, detergent solutions, and so forth as disclosed, for example, by U.S. Pat. No. 5,147,806.

Brief Summary Text (19):
It is therefore an object of the present invention to provide a novel, cost effective electrode and disposable for conducting ECL assays.

Brief Summary Text (25):
The invention relates to a cassette for conducting ECL reactions and assays comprising one or more binding domains immobilized on a support. The support may act as an electrode for generating electrochemiluminescence. Alternatively, one or more electrodes may be on additional supports, and said electrodes may be brought into proximity to the first support so as to generate ECL. The cassette may have one or more electrodes or one or more electrode/counterelectrode pairs. The cassette may also comprise a second support capable of being placed adjacent to the first support to provide sample containing means therebetween, and/or serve as an electrode. The binding domains are patterned on a support surface and are prepared so as to bind analytes or reagents of interest.

Brief Summary Text (26):
The invention further relates to novel, disposable electrodes amenable to use in a disposable format. These electrodes can be comprised of various forms of carbon such as glassy carbon, carbon black or carbon (graphitic) nanotubes.

Brief Summary Text (27):
The invention further relates to composite electrodes, i.e. electrodes comprised of more than one material. These electrodes can be tailored to control performance, cost and manufacturability to make them amenable to use in a disposable format.

Brief Summary Text (28):
The invention further relates to assays in which particles are used as solid-phase supports for binding reagents. Said particles are captured on a porous electrode by filtration and analytes are detected. Kits based on pre-prepared conducting filters with particles are described.

Brief Summary Text (29):
The invention further relates to electrodes that can be used to resolve two or more ECL signals. Methods for the modification of electrodes are also described.

Brief Summary Text (33):
The invention is also in rapid disposable electrochemiluminescence assays. Commercial ECL assays are performed using a flow cell with a working and counter electrode. A disposable electrode, as disclosed herein, does not require washing and/or cleaning to eliminate carry-over and regenerate a uniform electrode surface as does a permanent flow cell electrode.

Brief Summary Text (34):
The invention also provides for increased kinetics through the use of porous electrodes. Formatted and/or porous disposable electrodes are used to rapidly produce assay results. Assay results with disposable electrodes may be achieved in less than an hour. In preferred embodiments ECL assay results from disposable electrodes may be achieved in less than 30 minutes and in some cases less than 15 minutes. In the most preferred embodiments, the assay results can be achieved in less than 5 minutes or in the most advantageous case, than 1 minute. In multi-assay formats of the invention more than one ECL assay result may be achieved in such time periods or less. Kits for rapid disposable ECL systems are disclosed.

Brief Summary Text (35):
Additionally, the invention provides for portable ECL diagnostic instruments. Cartridges or kits for portable ECL diagnostics may use the novel disposable electrodes and reagent packs. PMAMS and electrodes for ECL assays may be packaged as kits for use in portable ECL instrument readers. Such kits and ECL instrument readers may be used to achieve assay results in short time periods. Assay results may be achieved in the very short time periods discussed above.

Drawing Description Text (2):
FIG. 1 illustrates a cassette according to the invention wherein a plurality of binding domains are present on an electrode.

Drawing Description Text (3):
FIG. 1A illustrates two supports forming a cassette according to the invention wherein a plurality of binding domains 14 are present on support 10 and a plurality of corresponding electrodes 16 is present on support 12 so that approximation of the supports places an electrode pair adjacent to each binding domain.

Drawing Description Text (4):
FIG. 2 illustrates two supports forming a cassette according to the invention wherein a plurality of binding domains 30 on support 26 are adjacent to each of single electrodes 32 so that approximating supports 26 and 28 places each of counterelectrodes 38 adjacent to each of binding domains 30.

Drawing Description Text (5):
FIG. 3 illustrates two supports forming a cassette according to the invention wherein a plurality of binding domains 48 have electrode counterelectrode pairs 50 adjacent thereto on support 44. Support 46 may optionally be placed adjacent to support 44 so that support 46 provides sample containing means adjacent to binding domains 48 and electrodes 50.

Drawing Description Text (10):
FIG. 6A illustrates the approximation of a multi-array of electrodes in register with a surface having patterned multi-array, multi-specific binding domains. A removable electrode protection barrier is shown between the electrode array and the binding surface array. The entire assembly comprises a cassette for conducting a plurality of ECL reactions.

Drawing Description Text (11):
FIG. 6B illustrates the approximation of an array of registered or aligned addressable working and counterelectrodes. The electrodes may be shape complementary with the binding domain or of other shapes (e.g., interdigitating).

Drawing Description Text (12):
FIG. 7 illustrates the side view of an approximated array of registered or aligned addressable working and counterelectrodes and the complementary binding surface wherein conducting polymers are grown from the surfaces of the electrodes across the gap between the electrode array and the binding domains so as to extend the potential field around the ECL label of the sample to increase the efficiency of the ECL reaction.

Drawing Description Text (14):
FIG. 9 illustrates the side view of an approximated array of registered or aligned addressable working and counterelectrodes and the complementary binding surface wherein the electrodes have fine projections extending into the gap between the electrode surface and the binding domains in order to extend the potential field around the ECL label of the sample, to increase the efficiency of the ECL reaction.

Drawing Description Text (16):
FIG. 11 illustrates the side view of a support having a metallic layer thereon to provide a single electrode and binding surface assembly in the form of a cassette. An array of self-assembled monolayers ("SAMs") is patterned on the metallic layer.

Drawing Description Text (17):
FIG. 12 illustrates the side view of a support having a metallic layer thereon to provide a single electrode and binding surface assembly in the form of a cassette. An array of SAMs is patterned on the metallic layer and conducting microparticles are shown interspersed among the patterned SAMs so as to extend the potential field around the ECL label of the sample, to increase the efficiency of the ECL reaction.

Drawing Description Text (18):
FIG. 13 illustrates the side view of a support having a metallic layer thereon to provide a single electrode and binding surface assembly in the form of a cassette. An array of self assembled monolayers or SAMs is patterned on the metallic layer and the growth of a conducting polymer and/or fiber from the ECL label so as to extend the potential field around the ECL label of the sample to increase the efficiency of the ECL reaction, is illustrated.

Drawing Description Text (19):
FIG. 14 is a diagram of a support having an array of electrode pairs controlled by a computer.

Drawing Description Text (20):
FIG. 15 is a diagram of a support having an array of electrode pairs.

Drawing Description Text (21):
FIG. 16 is a diagram of a support having an array of electrode pairs and computer system for controlling the energization of each electrode pair.

Drawing Description Text (22):
FIG. 17 is a diagram of a support having an array of electrode pairs and a computer system with a plurality of voltage sources and multiplexers for controlling the energization of each electrode pair.

Drawing Description Text (23):

FIG. 18 is a diagram of a support having an array of electrode pairs and a computer system with a plurality of switched voltage sources for controlling the energization of each electrode pair.

Drawing Description Text (24):
FIGS. 19(a)-(e) are plan views of several alternative electrode-counterelectrode pair combinations.

Drawing Description Text (37):
FIG. 30A illustrates cyclic voltammograms from electrochemical measurements on carbon fibril mat electrodes.

Drawing Description Text (38):
FIG. 30B illustrates cyclic voltammograms from electrochemical measurements on gold foil electrodes.

Drawing Description Text (41):
FIG. 33 demonstrates that the use of surfactants can reduce non-specific binding between ECL-TAG1-labeled protein and carbon fibrils.

Drawing Description Text (42):
FIG. 34 shows a schematic of a top view of an experimental cell used to measure electrochemical properties and ECL on a fibril mat electrode.

Drawing Description Text (43):
FIG. 35 shows an ECL signal obtained using a fibril mat as an electrode and 1000 pM TAG1 (solid line) in solution and a signal from assay buffer (no TAG1) (dashed line).

Drawing Description Text (44):
FIG. 36 shows a schematic of a two surface PMAMS device, in which two arrays of supported electrodes are separated by a patterned dielectric layer.

Drawing Description Text (45):
FIG. 37 illustrates an apparatus with a plurality of binding domains (3702) on one support and an electrode and counterelectrode on another support.

Drawing Description Text (46):
FIG. 38 shows a cassette where binding domains are presented on the surfaces of distinct objects supported on the counter electrode.

Drawing Description Text (51):
FIG. 43 demonstrates that fibril mats can be used as electrodes for ECL of Antibody-TAG1 adsorbed to the mats.

Drawing Description Text (52):
FIG. 44A shows ECL intensity of a TAG1 labeled protein immobilized on an electrode.

Drawing Description Text (53):
FIG. 44B shows the cyclic voltammogram of a coated electrode.

Drawing Description Text (55):
FIG. 45B shows the cyclic voltammogram of a coated electrode indicating partial preservation of the coating.

Drawing Description Text (57):
FIG. 46B shows the cyclic voltammogram of a coated electrode indicating substantial loss of the coating.

Drawing Description Text (59):
FIG. 48 shows the dose response for an AFP immunoassay that involves formation of a sandwich complex on streptavidin-coated Dynal beads, capture of the beads on a fibril mat electrode, and detection of the bound complex by ECL.

Drawing Description Text (60):
FIG. 49 shows the dose response for an AFP immunoassay that involves the formation of a sandwich complex on streptavidin-coated silica particles, the capture of the particles on a fibril mat electrode, and detection of the bound complex by ECL.

Drawing Description Text (62):
FIG. 51 shows the dose response for an AFP immunoassay that involves the formation of a sandwich complex on a streptavidin-coated SAM of alkanethiolates on a gold electrode, and detection of the bound complex by ECL.

Drawing Description Text (63):
FIG. 52 illustrates the presentation of TAG moieties to the working electrode in a "Two Surface" assay.

Drawing Description Text (66):
FIG. 55 shows the dose response for a DNA assay that involves hybridization of a biotin-labeled oligonucleotide to a TAG1 labeled oligonucleotide, capture of the complex on a streptavidin-coated fibril mat electrode and detection of the bound complex by ECL.

Drawing Description Text (73):
FIG. 62 shows a plot of the ECL signal (S-B, the difference between the ECL Signal (S) and the background signal (B)) as a function of the concentration of AFP (IU/mL) for an AFP assay. The ECL mediated AFP assay was conducted using plasma treated fibril-polymer composites as a support for binding reagents and as a working electrode.

Drawing Description Text (74):
FIG. 63 shows a plot of the ECL signal (S-B, the difference between the ECL Signal (S) and the background signal (B)) as a function of the concentration of AFP (IU/mL) for an AFP assay. The ECL mediated AFP assay was conducted using plasma treated fibril-polymer composites as a support for binding reagents and as a working electrode.

Drawing Description Text (75):
FIG. 64 shows a plot of the ECL signal (S-B, the difference between the ECL Signal (S) and the background signal (B)) as a function of the concentration of AFP (IU/mL) for an AFP assay. The ECL mediated AFP assay was conducted using plasma treated fibril-polymer composites (15% fibrils by weight) as a support for binding reagents and as a working electrode.

Drawing Description Text (76):
FIG. 65 shows a plot of the ECL signal (S-B, the difference between the ECL Signal (S) and the background signal (B)) as a function of the concentration of AFP (IU/mL) for an AFP assay. The ECL mediated AFP assay was conducted using plasma treated fibril-polymer composites as a support for binding reagents and as a working electrode.

Drawing Description Text (77):
FIG. 66 shows a plot of the ECL signal (S-B, the difference between the ECL Signal (S) and the background signal (B)) as a function of the concentration of AFP (IU/mL) for an AFP assay. The ECL mediated AFP assay was conducted using plasma treated fibril-polymer composites as a support for binding reagents and as a working electrode.

Detailed Description Text (3):
The cassette may include a plurality of electrodes able to selectively trigger ECL emission of light from ECL labeled reagents bound to the binding domains. FIG. 47 shows an multi-array ECL apparatus using a cassette 4700 which comprises a housing 4717, electrical connections to the electrode in the cassette 4718, a waveform generator or potentiostat 4719, a CCD camera for imaging the ECL emitted from the PMAMS 4720, and a microcomputer for controlling the waveform generator and analyzing the image received by the camera 4721.

Detailed Description Text (4):
In the embodiment of the invention shown in FIG. 1, a cassette 180 comprises a working electrode comprising a conducting material 181 on a support material 182. A plurality of binding domains, i.e. a PMAMS 183 are present on the electrode 181. The cassette also includes a means for introducing samples and reagents (fluid channel 184) and a counter electrode 185. A reference electrode 186 may also be included.

Detailed Description Text (5):
In another embodiment, a plurality of working electrodes are used to simultaneously generate an ECL signal at a plurality of binding domains. In this embodiment, the ECL signal from each binding domain is identified without the use of light imaging equipment.

Detailed Description Text (12):
The invention provides for ECL assay methods for detecting or measuring an analyte of interest, comprising (a) contacting one or more binding domains immobilized on an electrode, in which said contacting is with a sample comprising molecules leveled to an ECL label, (b) applying a voltage waveform effective to trigger ECL at said binding domains, and (c) measuring or detecting ECL.

Detailed Description Text (14):
The invention also provides ECL assay methods for detecting or measuring an analyte of interest, comprising (a) contacting one or more binding domains, said binding domains being immobilized on a surface of one or more supports, in which said contacting is with a sample comprising molecules linked to an electrochemiluminescent label; (b) bringing an electrode into proximity to said binding domains; and (c) applying a voltage waveform effective to trigger ECL at said binding domains; and detecting or measuring ECL.

Detailed Description Text (15):
In another embodiment, the invention provides ECL assay methods for (a) contacting one or more binding domains, said plurality of binding domains (i) being immobilized on a surface of one or more supports, and (ii) being spatially aligned with and in proximity to a plurality of electrode and counterelectrode pairs, in which said contacting is with a sample comprising molecules linked to an electrochemiluminescent label; (b) bringing an electrode and counterelectrode into proximity to said binding domains; (c) applying a voltage waveform effective to trigger electrochemiluminescence at said binding domains; and (d) detecting or measuring electrochemiluminescence.

Detailed Description Text (21):
In a specific example of the method of the invention shown in FIG. 20, a sandwich assay is conducted on a support (5) with a plurality of binding domains (BD) on its surface that are specific for binding a particular analyte (An). When a sample suspected of containing the analyte is applied to the binding domains, the analyte is bound to the binding domains. Antibodies (Ab), which are suitable for selectively binding analyte (An) and have been labeled with an ECL moiety (TAG) to form Ab-TAG, are then applied to the analyte on the binding domains. After excess, unbound Ab-TAG is washed off the binding domains, a potential waveform suitable for triggering electrochemiluminescence is applied to the TAG by electrodes (not shown)

to trigger an ECL emission from any TAG on the binding domains. The ECL signal is detected by light detection means and recorded by digital computer means.

Detailed Description Text (28):
The surface of the support on which PMAMS are prepared may contain various materials, e.g., meshes, felts, fibrous materials, gels, solids (e.g., formed of metals) elastomers, etc. The support surface may have a variety of structural, chemical and/or optical properties. For example, the surface may be rigid or flexible, flat or deformed, transparent, translucent, partially or fully reflective or opaque and may have composite properties, regions with different properties, and may be a composite of more than one material. The surface may have patterned surface binding regions and/or patterned regions where catalyses may occur according to the invention on one or more surfaces, and/or an addressable array of electrodes on one or more surfaces. The surfaces of the supports may be configured in any suitable shapes including planar, spheroidal, cuboidal, and cylindrical. In a specific embodiment, the support bearing a PMAMS is a dipstick.

Detailed Description Text (37):
PMAMS can be generated on the surface of matrices. Matrices may be highly conducting, e.g., metal electrodes or conducting polymer films; or matrices may be insulators; or semi-conducting and/or of medium conductivity. The matrix material may be an ionic conductor or a porous material. Such porous materials may be utilized as support material and/or a conductive material and/or a filter material and/or a channelling material (e.g., allowing passage of fluids, ionic species etc.).

Detailed Description Text (38):
The porous material may be combined with additional materials. For example, composite structures may be fabricated of porous materials with additional porous materials, conductive materials, semiconductive materials, channelling structures and/or solutions (e.g., ionic fluids). Such composites may be laminar structures, sandwich structures, and/or interspersed composites. A solid matrix may be used which is a porous material supported on a metal electrode. Alternatively, a porous material is sandwiched between conducting materials, semiconducting materials or a combination of semiconducting and conducting materials. One or more binding domains may be contained on one continuous slab of the porous material and/or may be located on a plurality of discrete objects on the support each with one or more binding domains. The porous material (e.g., gel) surface may be flat, hemispherical or take on any regular or irregular shape and/or may have a variety of physical properties (e.g., elastomeric, rigid, low density, high density, gradient of densities, dry, wet etc.) and/or optical properties (e.g., transparent, translucent, opaque, reflective, refractive etc.) and or electrical properties (e.g. conductive, semiconductive, insulating, variably conductive, for example wet vs. dry etc.). The porous material may be a composite of more than one materials.

Detailed Description Text (40):
The pores may extend partially and/or fully through the material or may be part of a network of pores. These pores may have dimensions ranging broadly from 50 .ANG. to 10000 .mu.m. In a preferred embodiment, the material has some pores with dimensions ranging from 200 .ANG. to 500 .ANG. and some pores with dimensions ranging from 0.5 .mu.m to 100 .mu.m.

Detailed Description Text (41):
The porosity of the material may be constant throughout the material or may increase or decrease as a function of the position in the material. The material may have a wide variety of pores of different size distributed in a disorganized and/or random manner.

Detailed Description Text (42):
For example, the material may have some pores that are large enough to pass objects

as large as biological cells, some pores that can pass biological media as large as proteins or antibodies, some pores that can pass only small (<1000 molecular weight) organic molecules, and/or combinations thereof.

Detailed Description Text (44):
The porous material may be able to support a current due to the flow of ionic species. In a further refinement, the porous material is a porous water-swollen gel, for example polyacrylamide or agar. A variety of other gel compositions are available (for example see Soane, D. S. Polymer Applications for Biotechnology; Soane, D. S., Ed.; Simon & Schuster: Englewood Cliffs, N.J., 1992 or Hydrogels in Medicine and Pharmacy, Vol. I-III; Peppas, N. A. Ed.; CRC Press: Boca Raton, Fla., 1987). Binding domains can be attached to matrices by covalent and non-covalent linkages. (Many reviews and books on this subject have been written; some examples are Tampion J. and Tampion M. D. Immobilized Cells: Principles and Applications Cambridge University Press: N.Y., 1987; Solid Phase Biochemistry: Analytical and Synthetic Aspects Scouten, W. H. Ed., John Wiley and Sons: N.Y., 1983; Methods in Enzymology, Immobilized Enzymes and Cells, Pt. B Mosbach, K. Ed., Elsevier Applied Science: London, 1988; Methods in Enzymology, Immobilized Enzymes and Cells, Pt. C Mosbach, K. Ed., Elsevier Applied Science: London, 1987; Methods in Enzymology, Immobilized Enzymes and Cells, Pt. C Mosbach, K. Ed., Elsevier Applied Science: London, 1987; see also Hydrogels in Medicine and Pharmacy, supra). For example, a protein can be attached to a cross linked copolymer of polyacrylamide and N-acryloylsuccinimide by treatment with a solution of the protein. The binding domains may also be integrated into a porous matrix in a step prior to polymerization or gelation. In one embodiment, binding domains may be attached to uncrosslinked polymers by using a variety of coupling chemistries. The polymers may then be crosslinked (for example using chemistries which include amide bonds, disulfides, nucleophilic attack on epoxides, etc.) (see for example: Pollack et al., 1980, J. Am. Chem. Soc. 102(20):6324-36). Binding domains may be attached to monomeric species which are then incorporated into a polymer chain during polymerization (see Adalsteinsson, O., 1979, J. Mol. Catal. 6(3): 199-225). In yet another embodiment, binding domains may be incorporated into gels by trapping of the binding domains in pores during polymerization/gelation or by permeation of the binding domains into the porous matrix and/or film. Additionally, binding domains may be adsorbed onto the surface of porous matrices (e.g., polymer gels and films) by nonspecific adsorption caused for example by hydrophobic and/or ionic interactions. Biotin may be advantageously used as a linking or binding agent. Avidin, streptavidin or other biotin binding agents may be incorporated into binding domains.

Detailed Description Text (45):
PMAMS can be generated on porous materials (e.g., gels) with varying pore size and solvent content. For example, polyacrylamide gels varying in pore size can be made by varying the concentration of acrylamide and the degree of crosslinking.

Detailed Description Text (46):
On such PMAMS with pore sizes smaller than the analyte, binding reactions will occur substantially on the surface of the gel. In this case, filtration and/or electrophoresis through the gel can be used to concentrate analytes at the surface of the gel and modulate the kinetics (e.g., increase the rate) of the binding reaction. Faster kinetics is advantageous in rapid assays (e.g., short times to results) and may generate increased sensitivity in a shorter time period.

Detailed Description Text (47):
On PMAMS with pore sizes larger than the analyte, binding reactions can occur on the surface as well as in the bulk of the gel. In this case, filtration can be used and/or electrophoresis can be used to increase the kinetics of binding and remove unbound species from the surface.

Detailed Description Text (55):

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Alternatively, drop(s) deposited on a surface contain reagents that can form a matrix. This matrix may be a solid, polymer or a gel. The formation of the matrix may be by evaporation of solvent. It may be by polymerization of monomeric species. It may be by cross-linking of preformed polymers. It may be by modulating temperature (e.g., cooling and/or heating). It may be by other methods. For example, a polymeric species may be cooled through a cooling transition or by addition of a reagent that causes gelling. The formation of the solid matrix may be induced by generation of reactive species at an electrode (including the substrate), by light (or other radiation) by addition of reagents that induce solidification or gelling, by cooling or heating. Additionally, the surface may contain catalysts capable of initiating matrix formation (e.g. gelling or polymerization).

Detailed Description Text (63):
Methods described to inhibit wetting or spread of applied reagents or sample on a surface as described in Section 5.13 infra, can also be used in the preparation of PMAMS (and/or in sample application). Applied potential (e.g., from the electrode/counterelectrode pair) may be used to further control the deposition and/or spreading of reagents and/or samples (see, e.g., Abbott et al., 1994, Langmuir 10(5):1493-1497).

Detailed Description Text (71):
The binding domains may be located on the working electrode and/or the counter electrode.

Detailed Description Text (72):
The different embodiments described herein for different types of PMAMS, supports, and electrodes and configurations thereof may also be practiced in combination with each other.

Detailed Description Text (88):
The voltage waveform (change in electrical potential/time) impressed upon the electrodes and counter-electrodes of ECL cells must be sufficient to trigger an ECL reaction. This voltage waveform usually is in the form of a uniform voltage sweep starting at a first voltage, moving steadily to a second voltage, moving back through the first voltage to a third voltage and then back again to the first voltage. For example, the waveform may start at a first voltage in a range from -0.5 through 0.5 volts, up to a second voltage in a range from 1 through 2.5 volts and moving back through the first voltage to a third voltage ranging from 0.0 to -1 volts. As another example, in simpler waves, the voltage can be modified from 0.0 to +3.5 to 0.0. The voltage waveforms may incorporate linear ramps, step functions, and/or other functions. The voltage waveforms may incorporate periods of time when the voltage remains fixed at one potential. The applied potential may be controlled relative to one or more reference electrodes, or, no reference electrodes may be used. Additionally, negative potential may be used. Thus, the voltages used to induce ECL emissions from the cassette of the present invention will be readily selected for optimal ECL signal intensity and specificity for the ECL label and assay medium.

Detailed Description Text (89):
In some applications, the voltage is preferably varied as the light emitted from the binding domain is measured. This is particularly important to determine the threshold value of the electrical field necessary to cause the binding domain to emit light. In this case, the electrical potential applied at the binding domain starts at a value believed to be below the threshold required to emit light, and a first measurement is made of the light emitted. If no light is measured, or the light is below a predetermined threshold, the electrical potential applied across the electrode pair is increased under computer control, such as by a computer controlled voltage source and another light measurement is made. This process can be repeated until the predetermined appropriate amount of light is received. In

this way, the voltage applied may be used as the assay signal.

Detailed Description Text (92):
The potential required for generating ECL may be generated by illumination of the working electrode surface if the working electrode is a semiconductor or contains another moiety that generates electrical current in response to light.

Detailed Description Text (93):
5.4. Addressable Electrodes and Methods for Using the Same

Detailed Description Text (94):
Numerous methods may be used for addressing the plurality of electrode/counterelectrode pairs. Several illustrative such techniques are illustrated in FIGS. 14-18. Shown in those figures by way of example are four electrode/counterelectrode pairs 101, 102, 103, 104 and a waveform generator which typically is a digital computer and which preferably is the same computer used for processing the ECL detected by the detection means.

Detailed Description Text (95):
In FIG. 14, each electrode/counterelectrode pair 101-104 is individually addressed by a pair of lines connected to the waveform generator. By way of example, lines 105, 106 access electrode/counterelectrode pair 101. An appropriate waveform may be applied by the waveform generator at any given time to any one or more of the pairs of lines connected to the various electrode/counterelectrode pairs.

Detailed Description Text (96):
To reduce the number of connections required to address the electrode pairs, alternatives to the direct connection scheme of FIG. 14 are provided. For example, a row-and-column accessing scheme is illustrated in FIG. 15 for electrically energizing some or all of the electrodes. In this scheme, one of the electrodes 201, 202 in each column of the plurality of electrode/counterelectrode pairs is connected to a common electrical conductor 205 on support 200, and each of the counterelectrodes in each row of the plurality of electrode/counterelectrode pairs is connected to conductor 207, 208 on the support 200. Conductors 205, 206 connect to connections C1, C2, respectively, at the edge of support 200 and conductors 207, 208 connect to connections R1, R2, respectively. Each of these connections is then connected by a separate line to the waveform generator. As a result, in the configuration of FIG. 15, the number of required connections and signal lines from the waveform generator has been reduced from 8 to 4.

Detailed Description Text (97):
To enable rapid and sequential energizing of each electrode pair, a computer controlled switching device is beneficial. The configuration of FIG. 16 shows a plurality of electrodes connected to a first multiplexer 310. A plurality of counterelectrodes are connected to a second multiplexer 320. The first multiplexer is also connected to a first pole of a voltage source 330 that typically supplies the time varying electrical potential described infra. The second multiplexer is also connected to a second pole of the voltage source. Using addressing lines A0-A3 electrically connected to each of the multiplexers and connected to latch 340, a computer processor 350 can direct the multiplexers to selectively connect any or all of the first electrodes to the first pole of the voltage source, and any or all of the second electrodes to the second pole of the voltage source.

Detailed Description Text (98):
As shown in FIG. 17, a plurality of voltage sources are connected through separate sets of multiplexers to each of the electrodes. If a first electrical potential or range of electrical potentials is required at a particular electrode pair, the multiplexers 410, 420 associated with the voltage source 430 providing that potential are addressed by the computer processor 350, typically through a latch 340, thereby connecting that particular voltage source to the electrode pair in

question. If a different electrical potential or range of electrical potentials is required for another electrode pair, the multiplexers 440, 450 associated with that different voltage source 460 are addressed by the computer processor, thereby connecting that voltage source through the associated multiplexers 440, 450 to the electrode pair.

Detailed Description Text (99):
If the electrode array in this embodiment has at least a portion of the electrode pairs independently driveable, as shown in FIG. 14, or 15, for example, one electrode pair can be driven by one voltage source while another electrode pair is simultaneously driven with another voltage source. Alternatively, the two voltage sources of FIG. 17 can be replaced with a single voltage source connected to both sets of multiplexers in parallel, allowing two electrode pairs to be driven from the same voltage source.

Detailed Description Text (100):
Instead of a duplicate set of multiplexers for each voltage source as shown in FIG. 17, a plurality of voltage sources 520, 530 can be provided as shown in FIG. 18. These voltage sources can be connected through a computer controlled electrical switch 510 or switches to a single set of multiplexers 310, 320. As shown in FIG. 18, the computer would direct switch 510 to connect a particular voltage source to the multiplexers, and would also direct the multiplexers (by signalling their address lines A0-A3) to connect the selected voltage source to the particular electrode pair desired.

Detailed Description Text (101):
Alternatively, the electrical potential applied to each of the electrode pairs in any embodiment can be varied. This is of particular benefit when a cassette having a plurality of different binding domains is used. Such a cassette may require a different range of applied electrical potential at different binding domains. Several different embodiments capable of varying the electrical potential applied to each electrode are contemplated.

Detailed Description Text (102):
Advantageously, a computer controlled voltage source may be used. A computer controlled voltage source is one that can be addressed by a computer to select a particular electrical potential to be supplied. Alternatively it can be programmed to sequentially apply a particular range of electrical potentials over a predetermined time. In such a system, address lines electrically connected to the computer and the voltage source would allow the computer to program the voltage source to produce the particular electrical potential to be applied to the electrode pair to be energized.

Detailed Description Text (103):
Additional methods for addressing the plurality of electrode pairs may also be used. For example, a plurality of reference electrodes may be placed in proximity to each of the plurality of electrode and counterelectrode pairs in order to sense the voltage applied thereto. In this way, additional control of the voltage waveform may be maintained.

Detailed Description Text (104):
FIG. 36 shows another embodiment of the invention; arrays of electrodes (3600, 3601) are supported on each of two surfaces (3602, 3603) separated by a pattern of gaps in an insulator 3604 (for example a plastic sheet with punched holes 3605. Each electrode may pass over a plurality of gaps. If a potential is applied between one electrode on each surface, current can only pass through a gap contacting both electrodes, thus limiting the location of any electrochemistry or ECL which may occur. In the preferred embodiment shown in the figure, the electrodes (3600, 3601) are arrays of lines on a support. The two sets of electrodes on the two surfaces are oriented perpendicular to each other. Gaps in the insulating sheet are located

only at the intersection of the electrodes from each surface.

Detailed Description Text (105):
This embodiment has the advantage over individually addressed electrode pairs that less electrical leads are required.

Detailed Description Text (106):
In an alternate embodiment, the insulator 3604 is omitted and the surfaces are placed in close proximity so that only a narrow gap exists between the two surfaces. In this embodiment, a potential applied between are electrode on each surface will preferentially cause current to pass at the intersection of the electrode (i.e., where the distance between the electrodes is minimal) thus limiting the location of any electrochemistry or ECL which may occur.

Detailed Description Text (108):
The light generated by the triggered ECL emission is detected by an appropriate light detector or detectors positioned adjacent to the apparatus of the invention. The light detector may be, for example, film, a photomultiplier tube, photodiode, avalanche photo diode, charge coupled device ("CCD") or other light detector or camera. The light detector may be a single detector to detect sequential emissions or may be plural to detect and spatially resolve simultaneous emissions at single or multiple wavelengths of emitted light. The light emitted and detected may be visible light or may be emitted as non-visible radiation such as infrared or ultraviolet radiation. The detector or detectors may be stationary or movable. The emitted light or other radiation may be conducted to the detector or detectors by means of lenses, mirrors and fiberoptic light guides or light conduits (single, multiple, fixed, or moveable) positioned on or adjacent to the binding surface of the cassette or the detector may receive the light directly. In addition, the supports, PMAMS and electrode surfaces themselves can be utilized to guide or allow transmission of light.

Detailed Description Text (118):
5.7. Preparation of Electrodes for Multi Arrays

Detailed Description Text (119):
The electrodes may be broadly from 0.001 to 10 mm in width or diameter. In a preferred range the electrodes are from 0.01 to 1 mm in dimension (width or diameter or widest dimension depending upon the geometry of the electrodes).

Detailed Description Text (120):
Preferably, the electrodes are fabricated from suitable conductive materials, such as transparent metal films or semiconductors (e.g., gold or indium-tin oxide, respectively), as is well known to the art, for example, for the fabrication of liquid crystal displays and the like. In the assembled form of the cassette, sufficient space remains between the first and second supports to contain an analytic sample as, for example, a thin film or a wetted surface.

Detailed Description Text (121):
The electrodes may be fabricated from materials that contain carbon, e.g. particulate carbon, carbon black, carbon felts, glassy carbon, carbon fibers, carbon fibrils and/or aggregates of the above.

Detailed Description Text (123):
By way of example, a fibril mat may be used as a working electrode, a counter electrode or a reference electrode in analytical and/or preparative electrochemistry. In one example, the fibril mat is used as an electrode for electrochemiluminescence (ECL).

Detailed Description Text (124):
The binding domains of the PMAMS may be supported by an electrode, e.g. a fibril

mat or an electrode formed from carbon black. The PMAMS of the invention has a plurality of discrete binding domains, of which two or more may be identical to each other or may differ. The fibril mat supports one or more binding domains.

Detailed Description Text (134):
The filter may be used to trap the fibrils in its pores and so form a mat in which the filter acts as a support. In FIG. 24, a fibril mat 2400 may be prepared by passing a slurry of fibrils 2401, delivered by a source 2402, between two large rollers 2403. In this process, which may be analogous to processes found in the fabrication of paper or polymer sheets, the rollers force the liquid from the suspension and a large, continuous mat of fibrils is produced from which smaller mats may be cut.

Detailed Description Text (139):
Suspensions of fibrils suitable for forming fibril mats by filtration may be formed by dispersing one or more carbon fibrils in an appropriate liquid, quasi-solid or gel. Examples of appropriate liquids include but are not limited to water, ethanol, methanol, hexane, methylene chloride, buffered solutions, surfactants, organic solvents, solutions of containing biological media (e.g., as proteins, antibodies or fragments thereof, cells, subcellular particles, viruses, nucleic acids, antigens, lipoproteins, liposaccharides, lipids, glycoproteins, carbohydrates, peptides, hormones or pharmacological agents, solutions of small molecules, polymer precursors, solutions of acids or bases, oils and/or combinations thereof).

Detailed Description Text (140):
A suspension of fibrils may be prepared by dispersing carbon fibrils in an aqueous solution by means of sonication. In another embodiment, surfactant and or detergent may be added.

Detailed Description Text (155):
Fibril mats may have pores. These pores may extend partially and/or fully through the mat or may be part of a network or pores. These pores may have dimensions ranging broadly from 50 .ANG. to 1000 .mu.m. In a preferred embodiment, the fibril mat has pores with dimensions ranging from 200 .ANG. to 500 .ANG.. The porosity of the mat may depend on the density of the mat, among other factors.

Detailed Description Text (156):
The porosity of the mat may be constant throughout the mat or may increase or decrease as a function of the position in the mat. The fibril mat may have a wide variety of pores of different size distributed in a disorganized and/or random manner.

Detailed Description Text (158):
The porosity of the mat may be varied by including different amounts of aggregates of carbon fibrils, where aggregates have different size, shape, composition, or combinations. In a particular example, a mat can be prepared from individual fibrils, CC fibrils (described supra) and BN fibrils (described supra), or different combinations. For example, the fibril mat may have some pores that are large enough to pass objects as large as biological cells, some pores that can pass biological media as large as proteins or antibodies, some pores that can pass only small (<1000 molecular weight) organic molecules, and/or combinations thereof.

Detailed Description Text (160):
The fibril mat is supported by or on another material. By way of example, the supporting material may be a metal, plastic, polymer, elastomer, gel, paper, ceramic, glass, liquid, wax, oil, paraffin, organic solid, carbon or a mixture of two or more of each. The material may be solid or liquid. If it is solid, it may contain one or a plurality of holes or pores. In specific examples, the support may be a metal mesh, a nylon filter membrane or a filter paper. The support may be a conductor, a semiconductor and/or an insulator. The fibril mat may incorporate

another material, for example thin fibers, shards, or balls of metal to increase the conductivity of the mat. In another example, the fibril mat may incorporate other carbon, glass and/or metal fibers of varying size, shape and density to create a different porosity than can be achieved with fibrils alone. In another aspect, the mat may incorporate magnetic beads (for example, DYNAL beads). In the latter example, the beads may either serve to change a property of the mat, or may themselves be used as supports to immobilize binding domains.

Detailed Description Text (168):
One or more chemical moieties that reduce or prevent non-specific binding may be present in, on, or in proximity to a carbon-containing electrode (e.g. carbon black) or one or more fibrils, one or more fibril aggregates, a dispersion of fibrils in another material and/or a fibril mat. Such moieties, e.g., PEG moieties and/or charged residues (e.g., phosphates, ammonium ions), may be attached to the electrode.

Detailed Description Text (169):
Materials used in the support, electrode and/or binding domain may be treated with surfactants to reduce non-specific binding. For example, fibrils or fibril mats may be treated with surfactants and/or detergents that are well known to one of ordinary skill in the art (for example, the Tween series, Triton, Span, Brij). The fibrils or fibril mats are washed, soaked, incubated with, sonicated in, and/or a combination thereof with solutions of surfactants and/or detergents. Solutions of PEGs and/or molecules which behave in similar fashion to PEG (e.g., oligo- or polysaccharides, other hydrophilic oligomers or polymers) ("Polyethylene glycol chemistry: Biotechnical and biomedical applications, Harris, J. M. Editor, 1992, Plenum Press) may be used instead of and/or in conjunction with surfactants and/or detergents.

Detailed Description Text (178):
The ECL TAG may contains charged residues that could be used to selectively attract a TAG-labeled moiety to a support and/or electrode. For example, a derivatized ECL TAG which has a net negative charge may have a relatively low affinity for an electrode at more reducing potentials and then have higher affinity for the electrode as the electrode potential becomes more oxidizing. The affinity of the ECL label and/or binding reagents to the electrode may be made to modulate. This modulation may be used to improve the kinetics of binding or improve the efficiency of a washing step.

Detailed Description Text (187):
The fabrication of metallic electrode patterns and conductive elements to distribute electrical current to such electrodes on a surface is carried out by methods well known to the art (see, e.g., Leventis et al., U.S. Pat. No. 5,189,549). The preparation of metal films on transparent surfaces is used to produce liquid crystal displays and is readily adapted to the preparation of electrodes according to the invention. Haneko, 1987, Liquid Crystal TV Displays, Principles and Applications of Liquid Crystal Displays, KTK Scientific Publishers, Tokyo, D. Reidel Publishing. Transparent electrode surfaces may also be prepared, for example, according to the method of DiMilla et al., 1994, J. Am. Chem. Soc. 116 (5):2225-2226. 0.5 nm of titanium and 5 nm of gold are deposited on transparent substrates (glass or plastic). A thin gold layer as prepared by the method of DiMilla, supra may be used to prepare a transparent electrical structure by the method of Kumar supra. Modifications of this procedure to increase the thickness of the conductor layers for improved current carrying capacity while preferably maintaining transparency are desirable and readily apparent to the ordinary artisan. Such techniques may be used to prepare electrode surfaces that are aligned with or in proximity with discrete binding domains of a PMAMS.

Detailed Description Text (188):
In addition, the films and/or monolayers may be composed of moieties which

facilitate the transfer of electrical potential from the electrode surface to the ECL label, rather than using insulating moieties (e.g., alkyl chains) as taught by Zhang and Bard. For example, pi orbital overlap in extensively conjugated systems can be used for electron transfer. Such pi orbital electron transfer is provided by poly-pyrole or other conjugated rings or double bonded structures.

Detailed Description Text (189):
Oligonucleotides may be utilized to modulate electron transfer. For example, overlapping pi bonds in double stranded DNA may be utilized to increase electron transfer rates. Oligonucleotides bound to an electrode surface can be utilized as a binding agent in a binding domain. Upon binding a complementary oligonucleotide sequence a double strand with organized overlapping pi bonds is formed. In a particular embodiment, a first or primary immobilized (e.g., covalently linked to a support) oligonucleotide is ECL labeled. In another embodiment a secondary complementary oligonucleotide or oligonucleotide of partially complementary sequence to the primary oligonucleotide is ECL labeled. A tertiary oligonucleotide complementary to or partially complementary to the secondary oligonucleotide is labeled (e.g., a sandwich assay). Branched oligonucleotide chains may also be utilized. A variety of oligonucleotides and/or oligonucleotide mimics can be utilized (e.g., oligonucleotides with modified bases and/or modified backbones containing for example nitrogen and/or sulfur). Differential studies may be performed. Variable stability of pi overlap in oligonucleotides and/or oligonucleotide complexes may be monitored through modulation of electron transfer. The signal (e.g., ECL light generated and/or impedance measurements) generated from a pi bond stabilized ECL labeled double helical oligonucleotide pair may be correlated against the signal and/or expected signal from a more disordered single stranded oligonucleotide. The variation in ECL signal between a fully complementary ECL labeled double stranded oligonucleotide and a partially complementary ECL labeled double stranded oligonucleotide may be correlated. Additionally, oligonucleotide complexes of multiple oligonucleotides may be utilized. For example, triple helices may be employed.

Detailed Description Text (190):
Modulation of electron transfer rates may be measured using ECL detection as well as electronic means. ECL labels may be covalently linked to oligonucleotide strands and/or non-covalently associated (e,g., intercalated). DNA may be coupled to the electrode without the use of a linker (e.g., adsorption of 5' thiolated DNA on gold) or with a short (<10 atom) linker to ensure low resistance to electron transfer from the DNA to the electrode. A linking chain may be used that can efficiently transport electrons from the electrode to the DNA strand (e.g., a polyacetylene chain).

Detailed Description Text (191):
A mixed monolayer and/or film may be used in which at least one constituent of the monolayer or film, as the case may be, facilitates the transfer of electrical potential. Alternatively, a molecule or particle that facilitates the transfer of electrical potential is adsorbed to the monolayer or film. As examples of the foregoing, the pi conjugated monolayers and/or conducting micro-particles which adsorb to and/or are approximate to the electrode surface, may be used. Patterned regular gaps are created in the monolayer and or film. By utilizing controlled patterns of gaps in an ordered substantially perpendicular SAM composed of long chain alkane thiols (i.e., insulating) to which ECL labels have subsequently been attached, the effective potential imposed at the ECL labels can be controlled. For example, FIG. 11 shows a cassette 1200 formed of a single support 1202 with a metallic layer 1204, a SAM pattern 1206 and gaps 1208 between the SAM patterns.

Detailed Description Text (192):
ECL labeled proteins may be non-covalently linked to a monolayer surface. An ECL labeled protein may adsorb to the surface of a methyl terminated alkane thiol derivatized gold surface. The gold surface may act as the working electrode or the

counter electrode. A plurality of binding domains may be incorporated on a single support as is illustrated in FIGS. 11-13. In preferred embodiments the binding domains contain labelled and/or unlabelled proteins and/or nucleic acids and/or cells and/or chemical species.

Detailed Description Text (196):
Further, an electric potential sufficient to release the components of the monolayer may be applied. It is desirable to release such monolayer components where the volume above the electrode surface is small (e.g., another support or plate resting on the electrode surface). In this way as the monolayer is disrupted, even some ECL labels that are not efficiently excited may be excited by the electrode surface to generate the electrochemiluminescent signal and the ECL labels are restricted to a small volume restricting diffusion from the electrode. Various monolayer compositions may be utilized to control the degree of monolayer disruption for a given potential. Monolayers with components with strong inter-component affinity will be more resistive to monolayer disruption. Longer alkane chain thiols resist disruption more effectively than short alkane chain thiols. By varying the chain length the desired stability may be achieved.

Detailed Description Text (197):
Modification of the binding domains within a PMAMS may be used to modulate the ECL signal. A series of voltage waveforms is applied so as to generate a multiplicity of ECL signals. Said multiplicity of ECL signals may be utilized to gain extra and/or better results. Statistical analysis of the rate of modulation of the ECL signal may be correlated to the overall quality of one or more binding domains. Additionally, said multiplicity of ECL signals may be utilized to increase signal to noise by, for example, filtering certain ECL signals of a series. Further, multiple electronic potential waveform pulses may be utilized to reduce undesirable modulation of signal due to non-specific binding. Electronic potential may be applied to prevent non-specific binding of certain charged species. Additionally, electronic potential may be applied so as to promote the localization near a binding domain(s) of certain analytes or chemical species of interest. The voltage waveform applied supplies large over-potential (e.g., higher potential than is required to generate ECL). Over-potentials may be utilized to modulate ECL signals in a voltage wave series or in a single voltage wave pulse. Additionally, over-potentials may be utilized to modulate the ECL reaction kinetics and/or modulate the binding potential chemically and/or physically. Further, one or more voltage waveforms and/or other electronic probes known to those skilled in the art may be utilized to assess and/or correlate and/or extrapolate information on the quality and/or electronic properties of an electrode(s).

Detailed Description Text (198):
Preferably, the efficiency of the ECL reaction may be enhanced by extending the working electrode surface area by providing additional conducting means in contact with the electrodes. Projections or extensions from the electrode (e.g., wires or whiskers) of conducting materials or conducting particles may be used to increase the electrode surface area, such that the electrical field and more closely approaches the ECL label. Alternatively, indentations or wells in the electrode structures may serve the same purpose.

Detailed Description Text (199):
In particular, conductive particles may fill the gaps on the electrode surface and/or cover the support or monolayer so that the electrical field around the ECL label is increased in absolute magnitude, as shown by FIG. 12. These conductive particles extend the electrode surface area and thereby increase the efficiency of the ECL reaction. FIG. 12 shows a cassette 1300 having a support 1302 bearing a patterned SAM 1306 on a metallic layer 1304 and indicates conducting micro-particles filling in the gaps (e.g., 1208 in FIG. 11) and extending above the metallic surface between the SAM patterns. For magnetic conducting particles, a magnet or magnetic field may be used to draw the particles to the surface. The

conductive particles may also be used as described to extend the electrical potential between the electrode surfaces and the binding domain of a PMAMS with two approximated supports. In FIG. 8, the cassette 900 consists of a first support 902 that has a multi array of electrodes, and a second support 904 that has a PMAMS. Conducting micro-particles 906 are positioned between the opposing surfaces in order to extend the electrical potential toward the ECL label on the binding domains (not shown).

Detailed Description Text (200):
Alternatively, conductive polymers are grown from the exposed gaps on the electrode surface to facilitate extending the electrical potential around the ECL label of the sample as shown by FIG. 13. FIG. 13 shows a cassette 1400 having a support 1402 bearing a metallic layer 1404 on a patterned SAM surface 1406. Conductive polymers 1408 are grown over the SAM surface to extend the electrical field provided by a multi array of electrodes (not shown) to binding domains (not shown) on the SAM surface. The conductive polymers may also be used as described to extend the electrical potential between the electrode surfaces and the binding domains of a PMAMS of two approximated supports as illustrated by FIG. 7. In FIG. 7, the cassette 800 consists of approximated supports 802 and 804. Conductive polymers 806 are grown between the opposed surfaces so as to extend the electrical potential toward the ECL label on the binding domain (not shown).

Detailed Description Text (201):
FIG. 9 illustrates a cassette 1000 formed with a first support 1002 having a multielectrode array, a second support 1004 having a PMAMS binding surface, wherein conductive projections (1006) (e.g., fine wire or other protrusions) of the working electrode extend the electrical field around the ECL label in the PMAMS binding domains.

Detailed Description Text (202):
The electrode pairs may be created in a variety of configurations. The simplest configurations, depicted in the figures accompanying this disclosure, are made of metal and/or metal oxide films and/or semiconductor films applied on a non-conducting planar surface. The electrodes of these electrode pairs preferably define between them a region of relatively constant width thereby providing a relatively constant electrical field.

Detailed Description Text (203):
Other configurations of the electrodes are provided. Several of these configurations are shown in plane views in FIGS. 19(a)-(e). FIG. 19(a) shows an inter-digitated comb-like electrode pair. In this structure, each electrode has a plurality of digits extending from the conductor making a comb-like shape. The electrode and counterelectrode pair may be positioned adjacent to a binding domain, or a binding domain may be positioned between an electrode and counterelectrode. FIG. 19(b) shows a pair of concentric electrodes, one circular and one semicircular. FIG. 19(c) shows two semicircular electrodes with their straight edges facing each other. FIG. 19(d) shows a pair of rectangular electrodes. FIG. 19 (e) shows a pair of interdigitated electrodes having complementary opposing curved surfaces to form a sinuous gap therebetween.

Detailed Description Text (204):
The electrode/counterelectrode pairs may also be formed into specific shapes complementary to shapes on the PMAMS binding surface for alignment purposes. Exemplary shapes are shown in FIG. 6B. A support 712 bearing electrode pairs 714-720 is shown. The electrode pairs may be, e.g., circular 714, interdigitated 716 triangular interdigitating 718 or multi electrode interdigitating 720.

Detailed Description Text (205):
In the embodiments shown in FIGS. 14-19 discussed supra, the electrode pairs are located on a single support. Alternatively, the electrode pairs are located on

first and second opposing supports as shown by FIG. 2.

Detailed Description Text (207):
Cassettes contain one or more supports of the invention. Cassettes may include a plurality of binding domains and one or more working electrodes.

Detailed Description Text (208):
FIG. 2 depicts a cassette where each of plural binding domains 30 on support 26 are adjacent to a different one of plural electrodes 32. Counterelectrodes 38 are formed on a second support 28. An ECL assay is conducted as previously described by placing a sample on binding domains 30 and then moving together supports 26 and 28 so that counterelectrodes 38 are each adjacent to each of binding domains 30 and an ECL reaction may be triggered as described above by waveform generator means 39, via a lead 34, and an ECL signal detected and recorded by light detector means 40, wire 41, and digital computer means 42.

Detailed Description Text (209):
FIG. 3 illustrates a cassette where each of plural binding domains 48 has a different one of plural electrode/counterelectrode pairs 50 adjacent thereto on support 44. Support 46 may optionally be placed adjacent to support 44 so that support 46 forms a sample containing means adjacent to plural binding domains 48 and plural electrodes 50. Thus, an ECL reaction may be triggered via electrical connection 52 by waveform generator means 54, and an ECL signal detected by light detector means 56 and recorded and analyzed by digital computer means 58.

Detailed Description Text (210):
A cassette is provided that contains one or more pairs of supports as shown in FIG. 21, each pair of supports being situated so that the surface of a first support 1501 that contains binding domains faces the surface containing binding domains on the second support 1502, in which each surface contains electrodes 1504 and binding domains 1506; such that each binding domain on the first support faces and is aligned with an electrode on the second support, and each binding domain on the second support faces and is aligned with an electrode on the first support.

Detailed Description Text (211):
FIG. 4 illustrates a cassette wherein ECL electrodes are optional. Binding domains 64 on support 60 are contacted with a sample suspected of containing an analyte. Regions 66 on support 62 contain reaction medium for detecting or measuring an analyte of interest or for carrying out a desired reaction. Support 60 and Support 62 are brought together so that binding domains 64 and regions 66 are contacted and the presence of an analyte or reaction product is determined by a reporter system, e.g. a calorimetric chemiluminescent or fluorescent signal that may be detected by photodetector means 68 and recorded and analyzed by digital computer means 70.

Detailed Description Text (212):
In a preferred embodiment, a cassette or apparatus of the invention comprises a means for sample delivery onto the plurality of discrete binding domains (see, e.g., element 1 on FIG. 1 of U.S. Pat. No. 5,147,806; element 1 on FIG. 1 of U.S. Pat. No. 5,068,088; each of which is incorporated by reference in its entirety). The means for sample delivery can be stationary or movable and can be any known in the art, including but not limited to one or more inlets, holes, pores, channels, pipes, microfluidic guides (e.g., capillaries), tubes, spigots, etc. Fluids can be moved through the system by a variety of well known methods, for example: pumps, pipettes, syringes, gravity flow, capillary action, wicking, electrophoresis, pressure, vacuum, etc. The means for fluid movement may be located on the cassette or on a separate unit. The sample can be placed on all of the binding domains together. Alternatively, a sample may be placed on particular binding domains by a capillary fluid transport means. Alternatively, samples may be placed on the support by an automatic pipetter for delivery of fluid samples directly to the PMAMS on a support, or into a reservoir in a cassette or cassette holder for later

delivery directly to the binding surface.

Detailed Description Text (215):
The plurality of binding domains and the plurality of electrodes/counterelectrodes on the supports are typically placed in registered proximity to one another by mechanical means, e.g., by using guide posts, alignment pins, hinges (between each support) or guide edges. Optical guide means may be used to position both supports and electronic means utilizing optical guide marks defined on the supports. Other systems using electrical or magnetic registration means are also available.

Detailed Description Text (216):
The supports of the cassette may be configured so as to protect the electrode pairs from contact with the sample until required to trigger an ECL reaction. For example, the electrodes may be kept separate from a binding domain surface until electrode contact with the sample is required by using various mechanical means such as a removable electrode protective means.

Detailed Description Text (217):
A cassette or apparatus of the invention comprises reference electrodes, e.g., Ag/AgCl or a saturated calomel electrode (SCE).

Detailed Description Text (219):
The cassette may also comprise more than two supports, with, for example, alternating layers of binding domains and electrodes or multiple supports comprising both a binding surface and an electrode surface on a single support. This will form a three dimensional array of ECL analysis cells. All of the foregoing components of the cassette are transparent, except, optionally, some areas between the binding domains. For example, multiple transparent binding surfaces, electrode surfaces, and supports may be stacked.

Detailed Description Text (220):
The first and second supports may be flat and opposed to define a sample-holding volume therebetween. Alternatively, the first and second support layers may be configured in other suitable shapes including spheroidal, cuboidal, cylindrical, provided that the two supports, and any other components thereof, conform in shape. For example, FIG. 10 shows a cassette 1100 formed from two adjacent non-planar supports 1102 and 1104. Each support has a surface complementary to the other in conformation. Either support may have a PMAMS surface or a multi electrode array or both. One or both of the supports may be elastomeric so as to conform to the shape of the other support. The supports or the cassettes may also be prepared in a precut format, or dispensed in a suitable length from a roll dispenser. The cassette may further include sample receiving means such as a sample-holding volume and sample distribution grooves, channels, indentations and the like.

Detailed Description Text (221):
FIG. 37 shows a cassette where binding domains (3702) in and/or on a matrix (3703) are presented on a surface (3701). A second surface (3700) supporting a working electrode (3704) and a counter electrode (3705) is placed so that the binding domains are in close proximity to the working electrode. Under conditions which lead to light generation from ECL label bound to the binding domains, light may be detected through either or both surfaces. An array of light detectors (3706, e.g., a CCD array, an intensified CCD array, or an avalanche photodiode array) is used to simultaneously measure the plurality of light signals from each of the binding domains. The light detector array images the light generated from binding domains. Lenses, reflectors and/or optical guides may be utilized to enhance imaging. In other examples, light detected from zones or regions of light detectors (e.g., a light detecting pixel(s)) is correlated to a binding domain(s). Image analysis may be used to aid in the correlation of detected light with binding domains. In one favored embodiment, the surface is elastomeric or compliant and therefore capable of making intimate contact with the electrode surfaces. The binding domains are

linked to polymers capable of carrying ionic currents from the counter electrode to the working electrode. In a more favored embodiment, the objects are water-swollen polymers capable of carrying an ionic current from the counter electrode to the working electrode.

Detailed Description Text (222):
FIG. 38 shows a cassette where binding domains (3805, 3806, 3807) are presented n the surfaces of distinct objects (3808, 3809, 3810) supported on the counter electrode (3800). A working electrode (3801) is placed in proximity to the surface of the objects. Under conditions which lead to ECL from TAGged groups bound to the binding domains, light may be detected through either or both of the electrodes (if one or both of the electrodes is transparent or semi-transparent) and/or from the side. An array of light detectors (3802) is used to simultaneously measure the plurality of light signals from each of the binding domains. The objects may be elastomeric and/or compliant and are therefore capable of forming intimate contact with the working electrode. The objects may be polymers capable of carrying ionic currents from the counter electrode to the working electrode. The objects may be water-swollen polymers capable of carrying an ionic current from the counter electrode to the working electrode.

Detailed Description Text (223):
A transparent support containing one or more binding domains is brought into contact with a carbon electrode, e.g. a fibril mat electrode or an electrode comprised of carbon black or carbon felt. Reagents may be flowed either between the support/binding domains and the fibril mat, or through the mat to the binding domains. Light may pass from the binding domains, through the transparent support to a detector.

Detailed Description Text (224):
In another preferred embodiment, an electrode is coated with an optically translucent or transparent layer of carbon (e.g. fibrils) so as to increase the effective surface area of the electrode.

Detailed Description Text (227):
In one embodiment, the PMAMS on supports, and cassettes containing the same, are designed to be inserted into an apparatus, that contains means for applying one or more test samples onto the PMAMS binding domains and initiating a plurality of ECL reactions. Such apparatus may be derived from conventional apparatus suitably modified according to the invention to conduct a plurality of ECL assays based on a support or cassette. The invention provides various apparatus adapted to carry out ECL assays using each of the specific embodiments of PMAMS described in the Sections hereinabove. An apparatus for conducting ECL reactions is disclosed by Zoski et al. (U.S. Pat. No. 5,061,445). Modifications required include the provision for support and/or cassette handling, multiple sample delivery, multiple electrode addressing by a source for a voltage waveform and multiple ECL signal acquisition and processing.

Detailed Description Text (228):
Elements of illustrative apparatus in accordance with the invention are shown in FIG. 6A. Such apparatus 700 comprises upper and lower supports 702, 704 and an electrode guard 710. The upper support bears a plurality of electrode/counterelectrode pairs (not illustrated). The lower support bears the binding domains 706. The apparatus is capable of removing the electrode guard from the cassette and positioning the electrode/counterelectrodes to contact the analyte bound to the binding domains. A reagent or fluid flow space 708 is adjacent to the support bearing the binding domains. The apparatus is also capable of simultaneously or sequentially sending an identical or individually determined voltage wave to each of the plurality of electrode/counterelectrode pairs to trigger ECL reactions in the cassette and then measuring the emitted ECL radiation, by a photon detector, e.g., light detector means. The apparatus can further

comprise temperature control means for maintaining the temperature of the support and/or cassette, or the environment thereon and adjusting the temperature as needed to optimize ECL reaction conditions. Temperature control means are preferably heating and cooling means, e.g., electrical resistive heating elements, cooling fans, refrigeration means, and any other suitable source of heating or cooling. Temperature control means also includes temperature sensors, e.g., a thermostat or thermocouple device, and means to turn the heating or cooling means on or off in response to detected temperature changes.

Detailed Description Text (230):
The apparatus also comprises an electrode contact means able to electrically connect the array of separately addressable electrode connections of the cassette to an electronic voltage waveform generator means, e.g., potentiostat (see e.g., FIG. 5 of U.S. Pat. No. 5,068,088). The waveform generator means delivers signals sequentially or simultaneously to independently trigger a plurality of ECL reactions in the cassette.

Detailed Description Text (231):
During an ECL assay, ionic current between working and counter electrodes may flow through tonically conducted liquid (for example water containing ionic salts), through a thin film of such liquid, and/or through an ionically conducting solid matrix.

Detailed Description Text (232):
Thus, an apparatus for measuring electrochemiluminescence in a sample can comprise a plurality of cells for holding at least one sample, wherein a cell may be formed from one or more electrodes and one or more counterelectrodes and a first support that comprises a plurality of discrete binding domains. The electrodes and counterelectrodes can be provided on the surface of the first support or on the surface of a second support wherein the second support is in close proximity to the binding domains on the first support. The electrodes and counterelectrodes may occur in pairs. The cell may further comprise a plurality of sensing electrodes to sense the voltage adjacent to the working electrode. The cassette may further comprise a cell containing a reference electrode.

Detailed Description Text (233):
The apparatus further comprises light detection means able to detect ECL reactions conducted in the cassette, e.g., by one or multiple detector means. Such detector means include, simply by way of example, an array of fiberoptic channels in register with the electrode array and positioned adjacent thereto, connected to an array of photodetector means, or to a single light detector means able to scan the array of ECL signals as emitted.

Detailed Description Text (236):
Alternatively, the apparatus comprises electrode translation means, for example, to scan one or more electrode/counterelectrode pairs across the binding surface to sequentially trigger ECL.

Detailed Description Text (244):
Additionally, the assay may be formatted so that the binding reagent attached to the multi-array multi-specific patterned surface is ECL labeled. Upon binding to certain analytes of interest in a sample, the ECL signal will be quantitatively modulated. For example, the ECL labeled binding reagent attached to the surface may be specific for an analyte on a cell surface e.g., antigens such as alpha and beta T cell antigen receptor antigens, or CD4 or CD8 antigens. Upon exposure to a mixture of cells, cells bound to the surface will sterically hinder the ability of an electrode surface, brought into proximity with the multi-array multi-specific surface, from exciting the ECL labeled binding reagent thus down-modulating the ECL signal.

Detailed Description Text (246):
Once the desired steps of contacting the binding reagents with analyte or competitor thereof and any binding partners thereto, have been completed, one then ensures that the ECL label is subjected to an environment conducive to ECL. Suitable ECL assay medium are known in the art. Such an assay medium advantageously includes a molecule that promotes ECL of an ECL label, including but not limited to oxalate, NADH, and most preferably tripropylamine. Such a "promoter" molecule can be provided free in solution, or can be provided by prior linkage to or by production at (e.g., as a product of a chemical reaction) the PMAMS surface, a monolayer on the surface, the binding domain, the electrode surface, a binding reagent, and/or an ECL label, etc. If the medium surrounding the ECL label bound to the binding domains resulting from the contacting steps is conducive to ECL, no changes to the medium need be made. Alternatively, the medium can be adjusted or replaced to provide a medium conducive to ECL. An electrode and counterelectrode is already proximate to the binding domain, or is brought near or in contact with the binding domain, a voltage waveform is applied, and ECL is detected or measured.

Detailed Description Text (247):
In a preferred embodiment of the invention, the above-described steps of contacting the binding reagents with analyte or competitor thereof and any binding partners thereto, are carried out in the absence of electrodes and counterelectrodes, i.e., such that the sample does not contact the electrode or counterelectrode. Subsequent to these contacting steps, electrodes and counterelectrodes are brought sufficiently close to the ECL label bound to the binding domain, to trigger an ECL reaction.

Detailed Description Text (261):
The fibril mats may be patterned such that there are a plurality of discrete hydrophobic and/or hydrophilic domains surrounded by hydrophilic and/or hydrophobic domains. Drops of aqueous solutions containing binding reagents may rest on hydrophilic regions and be confined by surrounding hydrophobic regions. These drops may contain, for example, fibrils, aggregates of fibrils, binding reagents, ECL reagents, reagents for assays, surfactants, PEGs, detergents, a plurality of biological molecules mentioned above by example, and/or combinations thereof.

Detailed Description Text (264):
The optical opacity of a material used for a support, electrode and/or binding domain may be varied to achieve desired properties. Such a material may be translucent, transparent or substantially opaque, depending on the thickness, compositing and/or optical density of the material.

Detailed Description Text (269):
Porous materials used in supports and/or electrodes may have more than one layer in which the upper layer has binding domains and other layers within the mat do not have binding domains. In one example, a fibril mat, (illustrated schematically in FIG. 29), the upper layer 2900 is thick enough to prevent passage of light that originates in layer(s) 2901, 2902 from the mat below this layer. Light 2903 that originates from sources 2904,2905 bound to this upper layer can be detected by a detector 2906 located at or in proximity to the surface of the mat. Light originating from sources 2907, 2908, 2909 in lower layers 2901, 2902 is absorbed and/or scattered by either or all layers and cannot be detected by the detectors 2906, 2910.

Detailed Description Text (271):
A porous material (e.g. a fibril mat) may act as the support for the binding domains, an electrode which may be used for ECL or other electrochemical applications, a filter that can be used to control delivery of reagents, and/or an optical filter that can transmit, absorb and/or scatter light to varying degrees.

Detailed Description Text (274):

The two electrode surfaces which are the active region of the pixel are on two surfaces facing each other in a sandwich configuration. The electrode surfaces are coated with, for example, complementary electrochromic materials. To reduce cross-talk a conductive electrolytic film is placed between the electrode surfaces with non-conductive regions between different electrode pairs (i.e., between pixel elements). If the coated electrode surfaces are hydrophilic then the areas of the surface around the electrodes are made to be hydrophobic (e.g., by means of stamping or deposition through a mask) and hydrophilic conductive droplets are placed on the electrode on the first surface (e.g., by means of a fluidics array) and then the second surface is robotically aligned and brought into contact with the first surface so that the electrodes are in register. The electrolytic droplets can thus be constrained to the area within one pixel without any conductive material between pixels. The electrode pairs of a pixel are side by side in close proximity on the same surface. If the coated electrode pairs are hydrophilic the area encompassing both electrodes is made to be hydrophilic with a hydrophobic ring around the hydrophilic electrode area (e.g., by means of stamping or deposition through a mask). The droplets described in the two embodiments above are stabilized using hydrophobic solutions. The viscosity of the solutions may be increased to increase the stability of the droplet arrays. The hydrophilicity and hydrophobicity may be reversed. In other embodiments the droplets may contain solutions capable of polymerizing to increase the stability and/or conductivity (e.g., conducting polymers) of the film between or above the electrode pairs. Additionally, structural features may be utilized to limit cross-talk between pixels. For example, an elastomeric stamp (e.g., poly(dimethylsiloxane)) with ring shaped stamp protrusion features capable of circumscribing side by side electrode pixel pairs on a surface may be used to isolate electrolytic solutions, gels, or films between pixels. Alternatively, side by side electrode pixel pairs may be placed in electrically insulating well-like structures on a surface, electrolytic solutions, gels or films placed in the wells above the electrodes, and the entire surface covered or coated to isolate and contain the electrolytic components of each pixel.

Detailed Description Text (284):
5.14. ECL Assays Employing the Capture of Particles on Porous Electrodes

Detailed Description Text (285):
The invention includes a method for performing an electrochemiluminescence binding assay in which a complex is formed. The complex includes, at least, a particle and a label compound capable of electrochemiluminescence. The complex may also include ligands used in electrochemiluminescence assays as disclosed for example in Yang H. J. et al., BioTechnology, 12, (1994), 193-194. The method includes the steps of (a) forming the complex; (b) collecting the complex by filtration on a porous, conductive electrode; (c) inducing the label compound in the collected complex to luminesce by imposing a voltage on the electrode; and (d) detecting the emitted luminescence from the electrode.

Detailed Description Text (286):
In another method for performing an electrochemiluminescence binding assay, the particle capable of complexing with a component of an electrochemiluminescence assay is first collected on a porous conductive electrode. Then the sample containing the analyte of interest is passed through the porous, conductive electrode and forms complex on the particle theretofore collected on the electrode. Then the label compound is induced to luminesce by imposing a voltage on the electrode and the emitted luminescence is detected to measure the presence of the analyte of interest. In a preferred embodiment the porous, conductive electrode will be pre-prepared with particles incorporated therein and upon use the sample containing analyte of interest will be passed through the electrode to form the complex.

Detailed Description Text (287):

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The invention can be adapted to methods for performing a plurality of electrochemiluminescence binding assays for a plurality of analytes of interest. In such assays a plurality of complexes are formed, each of the complexes including at least a particle and a label compound and these are collected on a plurality of discrete domains, each of the domains including a porous conductive electrode. As described, the particles of the label compound and optionally other assay components may be complexed in solution then collected on the domains or the domains may contain the particles in the first instance and be complexed with the label compound and optionally other assay components by passing the sample through the electrode.

Detailed Description Text (290):
The invention includes binding assays in which particles are used as solid-phase supports for binding reagents. The term particle implies no restrictions on the size, shape or composition. The particles are captured on a porous electrode by filtration and the presence of analyte is detected by the excitation of ECL from ECL-labels present in the binding complexes on the particles.

Detailed Description Text (292):
Highly sensitive and precise assays have been conducted using a system that employs a magnetic field to capture magnetic particles on a metal surface (see PCT published application WO92/14139; Deaver, D. R., Nature 377, (1995) 758-760; Yang, H. J. et al., BioTechnology 12, (1994) 193-194). This capture process places the particles in close proximity to an electrode so that excitation of labeled particles can be effected. This technology has been highly successful in many areas. It does, however, have some limitations (primarily cost and complexity) that restrict its use in low cost assays employing disposable cartridges.

Detailed Description Text (293):
A system that captures particles by filtration through porous electrodes takes advantage of the high binding capacity and favorable kinetics of particle-based assays. It also simplifies the fluidics, may employ a large variety of inexpensive, non-magnetic, commercially available particles, and can use inexpensive, porous, carbon-based electrodes. It can also improve the efficiency of ECL excitation of labels bound to particles. A porous electrode may have a significantly higher effective surface area than a non-porous electrode e.g., a metal film. If the electrode is a fibril mat, which is both porous and comprised of fibrous materials, the fibrils may contact, e.g. by wrapping around or laying across, a substantial fraction of the particle.

Detailed Description Text (294):
The invention includes a cassette containing a porous electrode that captures particles for the detection of analytes by ECL. The cassette may contain a working electrode comprising a thin ECL-active layer of carbon fibrils supported on an ECL-inactive filter (see Sec. 5.1 and the references cited therein for a detailed description of carbon fibrils. See Sec. 5.7 for a detailed description of fibril mats). A separate chamber in the cassette contains streptavidin-coated particles and dried binding reagents, e.g. a biotin-labeled capture reagent and an ECL-tag labeled detection reagent. The cassette also provides a means for introducing a liquid sample to the chamber containing the particles, means for capturing the particles on the working electrode by filtration, a counter electrode and a reference electrode. The invention also includes associated systems for conducting ECL assays with the cassette, e.g. a housing, electrical connections to the electrodes in the cassette, a waveform generator or potentiostat, a charge coupled device (CCD) for imaging the ECL emitted from the PMAMS, and a microcomputer for controlling the waveform generator and analyzing the image received by the camera.

Detailed Description Text (295):
There are several desirable embodiments of the working electrode for particle-based assays in which the particles are captured by filtration. The material of which the

electrode is formed must be capable of exciting ECL from an ECL label in close proximity to it when an appropriate electrochemical potential is applied. If the electrode is porous, the size of the pores must be large enough to allow filtration of unbound reagents into or through the electrode but is sufficiently small to capture the particles.

Detailed Description Text (296):
Preferably, the working electrode is comprised of a conducting filter. Conducting filters may be formed, for example, from porous carbon, aggregate of particulate carbon, from mats of graphitic fibers, carbon fibrils and/or porous metals that are capable exciting ECL. The electrode may be composed of a non-conducting porous material, e.g., a commercially available polymer-based filtration membrane, coated with an ECL-active material such as gold, platinum and/or a mat of graphitic fibers). The electrode may have multiple layers. In one embodiment, a thin layer of an ECL-active electrode material is deposited on a thicker ECL-inactive (but electrically conducting) material. The terms "active" and "inactive" refer to the relative efficacy of the electrode for exciting ECL from an ECL label, this characteristic being dependent on both the structure of the ECL label and the conditions used to trigger ECL in a specific application. The conductive, ECL-inactive layer ensures good electrical contact along the entire surface of the active layer, but prevents the excitation of ECL from unbound ECL-tag labeled reagents that have filtered through the active layer. In a preferred embodiment, the ECL-active layer is a thin mat of carbon fibrils and the ECL-inactive support is stainless steel filter paper.

Detailed Description Text (299):
Materials are available with a variety of functional groups on their surface. This allows for the use of a wide spectrum of immobilization chemistries. In some assays, the analyte itself may act as a particle. For example, an assay for cells with a specific cell-surface antigen (or an assay to quantify the amount of a cell-surface marker in a population of cells) may be carried out by treating the cells with an ECL-tag labeled antibody against the antigen followed by filtration of the cells onto the porous electrode.

Detailed Description Text (300):
This invention may be used to conduct many different binding assays including those described in section 5.10. These include immunoassays and nucleic acid hybridization assays in both competitive and sandwich formats. Many of these assays detect a labeled analyte or binding reagent in proximity to an electrode. Conducting the binding reactions on particles in suspension using gentle mixing if necessary is advantageous because the kinetics of the binding reactions are particularly favorable (they can approach those of a homogeneous reaction).

Detailed Description Text (301):
Alternatively, the particles can be deposited on an electrode and the binding reactions carried out by flowing samples past the trapped particles. Particles bearing binding domains can be deposited on the electrode in a patterned array, i.e. a PMAMS, by a variety of methods disclosed in Sec. 5.1.

Detailed Description Text (302):
In one embodiment, the particles are deposited on an electrode in an array that corresponds to the pattern of a 96-well plate. This particle/electrode fixture can form the basis of a kit used for high-throughput ECL assays. A mask with holes in a 96-well pattern is pressed against the electrode such that the holes in the mask are aligned with the pattern of deposited particles. The walls of the holes define the walls of the wells; the electrode and particles define the bottom of the wells and the binding regions. Preferred kits may contain any number of holes that meet industry standards, (e.g. 96 or 384 holes for high throughput screening).

Detailed Description Text (303):

Particles bearing binding reagents may be deposited in a plurality of zones on an electrode. There may be two or more zones with particles bearing the same binding reagents. There may be two or more zones with particles bearing different binding reagents.

Detailed Description Text (305):
Alternatively, the particles may be deposited uniformly on the surface of the electrode, (i.e. not in a patterned array) and a fixture with holes may be pressed against the electrode to define the active area of the electrode.

Detailed Description Text (307):
Particles can be used to prepare a PMAMS for the simultaneous execution of one or more assays for one or more analytes. By way of example, a plurality of suspensions of particles can be prepared wherein each suspension comprises particles bearing immobilized capture reagents. A PMAMS is formed by applying microdrops of the suspensions, e.g by methods disclosed in Sec. 5.1 to defined regions on the working electrode.

Detailed Description Text (310):
5.15. ECL Assays Employing PMAMS on Electrodes

Detailed Description Text (311):
The invention includes a cassette containing a PMAMS formed directly on the surface of an electrode. The cassette contains a working electrode comprising a thin metal film on a support material. A plurality of binding domains, i.e. a PMAMS, are present on the surface of the metal film. The cassette also includes a means for introducing fluid samples and reagents over the surface of the electrode, and a counter electrode to allow for electrochemical excitation of ECL at the working electrode. A reference electrode may also be included for better control of the electrochemical potential at the working electrode. An apparatus for conducting ECL assays including a cassette which may comprise a housing, electrical connections to the electrodes in the cassette, a waveform generator or potentiostat, a CCD camera for imaging the ECL emitted from the PMAMS, and a microcomputer for controlling the waveform generator and analyzing the image received by the camera.

Detailed Description Text (312):
The formation of PMAMS directly on the working electrode has several advantages over previous ECL systems: The combination of the working electrode and the solid phase support for the binding assays into one unit greatly simplifies the manufacture and execution of ECL assays in a disposable format, allowing disposable assays to be produced at lower cost. A plurality of assays can be performed without the use of multiple ECL labels. The excitation of ECL from each of a plurality of assays can be conducted simultaneously by applying a potential to one working/counter electrode pair, all of the binding domains being located on the surface of the same working electrode). The use of the surface of a metal as the support for the PMAMS allows the use of well developed technology--e.g., the formation and patterning of self-assembled monolayers (SAMs) on metals--for the formation of the PMAMS.

Detailed Description Text (313):
The working electrode may be made of a wide range of materials including metals (e.g., gold and platinum), metal oxide conductors and semiconductors (e.g., ITO), carbon (e.g., graphite, carbon black, carbon fibrils), and conducting organic polymers (e.g., polythiophene). The electrode may comprise a composite of different materials.

Detailed Description Text (314):
In one embodiment, the working electrode is a thin (5 nm-10,000 nm) film on a substrate. The preparation of such films by techniques including evaporation, polymerization, sputtering, chemical vapor deposition, and plating is known in the

art. In a preferred embodiment, the working electrode is a thin film of gold evaporated on a substrate. The properties of the substrates for the thin film electrodes can be chosen according to the requirements of the assay system. The substrate may be solid, or if filtration of samples through the electrode or wicking of samples along the electrode is desired, the substrate may be a porous material e.g., a filtration membrane.

Detailed Description Text (315):
Binding reagents may be immobilized by non-specific adsorption directly to the electrode surface or by covalent attachment to a chemical functional groups on the surface of the electrode. One approach to the introduction of chemical functional groups on the surface of an electrode is electrodeposition or electropolymerization of thin films. Another approach is the preparation of self-assembled monolayers (SAMs). Examples of SAMs that can be prepared on electrode materials include monolayers of organic thiols on gold, and organic silanes on ITO (see Sec. 5.1). As shown in FIG. 50, a SAM may be prepared by the reaction of a molecule A--L--B 5032 with the surface of the electrode 5033, where A is the functional group responsible for the attachment of the molecule to the electrode, L is a linking chain, and B is a functional group which can be used for the attachment of binding reagents 5034 to the surface. Alternatively, B may be a binding reagent.

Detailed Description Text (317):
Many other chemistries for the immobilization of binding reagents are known in the art and can be employed. In some cases it may be desirable to control the density of functional groups B on the surface of the electrode in order to control the density of binding reagents immobilized on the surface or to maintain some desirable property of the surface, e.g. resistance to non-specific binding. The control of the surface density of the functional group B can be achieved by treating the electrode surface with a mixture containing the monomers A--L--B and A--L--C in a ratio determined to produce a mixed SAM with the desired concentration of B on the surface. The functional group C is chosen to be resistant to the immobilization chemistry used to couple binding reagents to B and may have other desirable properties such as producing surfaces with reduced non-specific binding.

Detailed Description Text (318):
The formation of PMAMS on the surface of an electrode can be achieved by a variety of methods including: (i) photolithographic immobilization; (ii) microcontact printing; and (iii) the controlled application of drops of binding reagents to the surface through the use of microcapillary arrays or ink-jet printing (see discussion in Sec. 5.1). Patterned SAMs can be used to better define the areas on the surface which are modified with binding domains. For example, microcontact printing can be used to pattern an area of circles on a gold surface presenting a hydrophilic SAM formed from a carboxylic acid terminated alkane thiol. The remaining gold surface can then be reacted with a methyl terminated alkane thiol to give a hydrophobic SAM. After activation of the surface with EDC in the presence of NHS, drops, each containing a different antibody, are applied to the hydrophilic circles. The drops will be confined to the hydrophobic regions due to the hydrophobic nature of the surface outside the circles, thus allowing careful control of the area of the immobilized binding domains.

Detailed Description Text (319):
The types of assays which can be conducted using PMAMS immobilized on a electrode include those described in Sec. 5.10. Many of these assays (for example, immunoassays and nucleic acid hybridization assays in both competitive and sandwich formats) rely on the detection of the binding to the electrode surface of a binding reagent or analyte that has been labeled with an ECL-active group (tag). The intensity of the signal emitted from a tag-labeled reagent on the surface of SAM can be strongly influenced by the nature of the potential waveform used to excite ECL. For example, SAMs of alkanethiolates on gold are good electrical insulators but highly oxidizing or reducing potentials at the electrode surface may reduce the

insulating properties of the film by introducing disorder in the monolayer. Excitation of ECL at potentials which do not introduce disorder into the SAM requires the transfer of electrons through the monolayer by tunneling. Much higher intensity signals can be achieved by applying potentials that introduce disorder into the SAM, thus allowing less hindered flow of current to the electrode. These potentials can be applied prior to or during the excitation of ECL. Alternatively, the SAMs could be formed using conditions known in the art to give disordered monolayers. The conductivity of monolayers can also be increased by including a constituent in the monolayer which facilitates the transfer of electrons (for example, by the introduction of a pi-conjugated system into the linking group L). The formation of SAMs with high conductivity is discussed in more detail in section 5.7.

Detailed Description Text (323):
FIG. 37 shows a cassette where binding domains 3702 in and/or on a matrix 3703 are presented on a surface 3701. After completion of binding reactions on the binding domains, a second surface 3700 supporting a working electrode 3704 and a counter electrode 3705 is positioned so that the binding domains are in close proximity to the working electrode. Luminescence from an ECL label bound to the binding domains may be detected from either or both surfaces. We refer to this configuration for ECL as the "Two Surface" ECL assay.

Detailed Description Text (324):
FIG. 38 shows a cassette where binding domains 3805, 3806, 3807 are presented on the surfaces of matrices supported on a counter electrode 3800. After completion of binding reactions on the binding domains, a working electrode 3801 is positioned in close proximity to the surface of the matrices. Luminescence from an ECL label bound to a binding domain may be detected through either or both of the electrodes if either or both of the electrodes is transparent or semi-transparent and/or from the side.

Detailed Description Text (325):
The invention also includes an apparatus for conducting ECL assays using cassettes containing a PMAMS. An apparatus for conducting ECL assays using the cassette described in FIG. 38 includes means for making electrical connections to the electrodes, means for controlling the potential at the electrodes, means for moving the matrix into close proximity with the working electrode and means for imaging the light emitted during excitation of ECL.

Detailed Description Text (326):
The Two Surface method has several advantages over previous ECL methods. A plurality of assays can be performed conveniently without the use of multiple ECL labels. The excitation of ECL from each of a plurality of assays can be conducted simultaneously by applying a potential to one working/counter electrode pair, all the binding domains being placed in proximity to the same working electrode. The working electrode can be protected during the binding reaction from the sample by a physical barrier that is removed prior to the excitation of ECL, thus, preventing contamination of the electrode surface which could result in a change in its electrochemical performance. As illustrated in FIG. 55 for the case of a sandwich immunoassay, the binding of ECL-tag labeled reagent 5203 to analyte 5202 bound to primary antibody 5201 immobilized on the matrix 5200 results in the optimal presentation of the tag 5204 to the electrode surface 5205, (i.e. with a minimum of organic material--such as protein, nucleic acid, or linking groups--between the tag and the surface of the electrode. The matrix may be used for the concentration and/or separation of components of a sample, for example, by electrophoresis and/or filtration through the matrix. The matrix may be used as a medium for the storage of assay reagents in dried or partially hydrated form. The surface of the matrix can be placed in conformal contact with the working electrode.

Detailed Description Text (327):

h e b b g e e e f c e e e ge

The PMAMS are preferably formed in and/or on a matrix with one or both of the following characteristics. The matrix is capable of carrying ionic currents between the working and counter electrodes, and therefore, can complete the electrochemical circuit. The matrix is preferably capable of making intimate contact with the working electrode e.g. it is elastomeric and/or compliant. Materials with these characteristics are known in the art and include porous materials such as filtration membranes and water-swollen polymeric gels. In some embodiments of the invention, e.g. if light excited at the working electrode is detected through the matrix, it is advantageous for the matrix to be transparent.

Detailed Description Text (328):
PMAMS can be generated on porous materials (e.g., gels) with varying pore size and solvent content. For example, polyacrylamide gels varying in pore size can be made by varying the concentration of acrylamide and the degree of crosslinking.

Detailed Description Text (329):
On such matrices with pore sizes smaller than the analyte, binding reactions will occur substantially on the surface of the gel. In this case, filtration and/or electrophoresis through the gel can be used to concentrate analytes at the surface of the gel and modulate the kinetics, e.g., increase the rate, of the binding reaction. Faster kinetics are advantageous in rapid assays and may generate increased sensitivity in a shorter time period.

Detailed Description Text (330):
On matrices with pore sizes larger than the analyte, binding reactions can occur on the surface as well as the bulk of the gel. In this case, filtration and/or electrophoresis can be used to increase the kinetics of binding as well as to remove unbound species from the surface.

Detailed Description Text (333):
The working electrode is preferably made from an electrode material that is capable of exciting ECL from an ECL label in close proximity to the surface when the appropriate electrochemical potential is applied. In some embodiments light is detected from the surface of the PMAMS through the working and/or the counter electrode. In these cases, it is advantageous to use a transparent or semi-transparent electrode material. These electrode materials are known in the art. Examples include films made of indium tin oxide as well as very thin (<30 nm) films of gold. Alternatively, it may be advantageous to protect the working electrode from the sample. A physical barrier on the electrode may protect it during incubation of the sample with the PMAMS. The physical barrier is then removed before placing the PMAMS in close proximity to the electrode.

Detailed Description Text (334):
5.17. ECL Assays Employing PMAMS on Composite Electrodes

Detailed Description Text (335):
In preferred embodiments of the invention the electrode is a composite of a polymer containing a multiplicity of carbon fibrils dispersed therein. Desirably the composite is porous.

Detailed Description Text (337):
An apparatus for detection of an analyte by electrochemiluminescence may comprise an electrode comprised of a composite of a matrix having a multiplicity of conducting particles dispersed therein and a binding domain containing a reagent capable of binding a component of an electrochemiluminescence assay. Desirably the matrix is a polymer and the conducting particles are carbon. The conducting particles are desirably are carbon fibers and best results obtained were the carbon fibers or carbon fibrils.

Detailed Description Text (338):

Apparatus for use in the detection of a plurality of analytes are also included in the invention. In such apparatus the electrode is comprised of a matrix containing a multiplicity of conducting particles dispersed therein and the plurality of binding domains supported on a surface of the electrode, each of those domains containing the reagent capable of binding a component of an electrochemiluminescence assay.

Detailed Description Text (339):
The properties of electrodes comprising a polymer and dispersed carbon fibrils may be modified by a subjecting the composite to various chemical and physical steps such as oxidation, exposure to a plasma and exposure to a reagent capable of derivatizing the electrode by addition of one or more functional groups. In the latter method the polymer can be derivatized or the fibrils contained therein can be derivatized or both can be derivatized. Desirably the composite is subjected to a chemical or physical treatment to affect the modification for a time sufficient to alter the electrical potential at which electrochemiluminescence occurs in an electrochemiluminescent compound situated at said composite. It is also within the invention to modify the properties of any electrode comprising a polymer and multiplicity of carbon fibrils dispersed therein by modifying the electrode to expose a desired functional group thereupon. The invention also includes electrodes which have been modified by chemical or physical treatment to alter the electrical potential at which electrochemiluminescence occurs.

Detailed Description Text (340):
The invention includes a cassette containing a PMAMS formed directly on the surface of an electrode comprised of more than one material, i.e. a composite electrode. The several components of such a cassette are described above.

Detailed Description Text (341):
The composite electrode may be comprised of conductive and/or electrochemically active particles impregnated in a support matrix. For example, the matrix may be comprised of oils, waxes, paraffins, plastics, ceramics, teflon, polymers, elastomers, gels and/or combinations thereof. Some examples of commercial polymers that can be used in the manufacture of composite electrodes include, but are not limited to, EVA, polyethylene (PE), polystyrene (PS), and ABS.

Detailed Description Text (343):
A composite electrode can be formed using any particles that when combined in a matrix provides an electrically conductive composite. The particles may be carbon, e.g. particulate carbon, carbon black, carbon fibers, carbon felts and preferably are carbon fibrils (Secs. 5.1 and 5.7).

Detailed Description Text (344):
Composites that contain more than one type of particle and/or more than one type of material for the matrix can be used. For example, a composite electrode may include one type of particle to impart electrical conductivity and ECL-activity and another type of particle as a support for binding domains.

Detailed Description Text (345):
In a preferred embodiment, a composite electrode is comprised of a blend of carbon particles and a matrix. In a particularly preferred embodiment, a composite electrode is comprised of carbon fibrils and a polymer. U.S. Pat. Nos. 5,304,326 and 5,098,771 describe polymer composites impregnated with fibrils.

Detailed Description Text (346):
Fibril-polymer composite electrodes can be produced by techniques known in the art of manufacturing plastic materials and parts. For example, flat electrodes can be cut from pressed sheets or extruded films of a fibril composite. Electrodes with complicated shapes or surface features such as grooves or channels for the movement of fluid or wells for reaction chambers can be formed by injection molding.

Detailed Description Text (347):
The composite electrode may be a solid or may be porous. Porous composites may be formed by using techniques for producing porous plastic materials, e.g., filtration membranes. Filtration through a porous composite electrode can improve the kinetics of binding reactions to binding domains immobilized on the surface of the electrode.

Detailed Description Text (348):
Binding reagents may be immobilized on unmodified composite electrodes. For example, binding reagents may be immobilized by non-specific adsorption onto the matrix and/or onto the conductive particles. Functional groups present on the matrix and/or the conductive particle can be used for immobilization of reagents. These reagents can serve as binding reagents and/or as reagents that change the properties of the surface e.g. wettability or resistance to non-specific binding. Methods for covalent and non-covalent immobilization of reagents on materials that can be used as matrices are known in the art (see Sec. 5.1).

Detailed Description Text (352):
The composite electrode may be modified by chemical or mechanical treatment to improve the immobilization of binding reagents. The surface may be treated to introduce functional groups for immobilization of reagents. Techniques that may be used include exposure to electromagnetic radiation, ionizing radiation, plasmas or chemical reagents such as oxidizing agents, electrophiles, nucleophiles, reducing agents, strong acids,and strong bases and/or combinations thereof (see Sec. 5.18).

Detailed Description Text (353):
One particularly interesting embodiment is the modification of such composite electrode, and more broadly a composite material (not limited to an electrode) comprising a matrix (such as a polymer) and one or more fibrils and/or fibril structures dispersed therein, by treatment with a plasma. The treatment is carried out in order to alter the surface characteristics of the fibrils, fibril structures and/or the matrix, which come in contact with the plasma during treatment; by this means the fibril composite treated can be functionalized or otherwise altered as desired. Once equipped with the teaching herein, one of ordinary skill in the art will be able to adapt and utilize well-known plasma treatment technology (without the need for further invention or undue experimentation) to the treatment of such composite materials. Thus, the treatment can be carried out in a suitable reaction vessel at suitable pressures and other conditions and for suitable duration, to generate the plasma, contact it with the composite material, and effect the desired kind and degree of modification. Plasmas such as those based on oxygen, ammonia, helium or other chemically active or inert gases can be utilized. Depending on its properties, the modified composition can be utilized as an electrode (such as described above) or for other applications.

Detailed Description Text (359):
It may be advantageous to immobilize binding reagents on both the matrix and the particles or it may be advantageous to immobilize binding reagents on only one of the components, i.e. the matrix or the particles. By way of example, a composite electrode comprised of fibrils in EVA (a copolymer or ethylene and vinyl acetate) may be treated with a mixture of chromic acid and sulfuric acid to introduce carboxylic acid groups on the electrode. These carboxylic acid groups can then be used to immobilize binding reagents containing amines by formation of an amide bond. Alternatively, a composite of fibrils in EVA can be treated with sodium hydroxide. In this case, the fibrils remain unmodified but hydroxyl groups are exposed on the polymer. These hydroxide groups can then be used to immobilize binding reagents containing a nucleophile.

Detailed Description Text (360):
Modification of composites may lead to other favorable properties. Modification of

the matrix and/or the particles may produce a composite electrode with a high binding capacity. The introduction of hydrophilic groups to the composite electrode may hydrate the matrix and lead to the formation of a thin water-swollen gel layer. Reagents can be immobilized within such a gel layer, allowing for the immobilization of more reagents than could occupy a flat, solid, surface with the same geometric surface area. Partial degradation of the matrix can increase the exposed surface area of the conducting particles and lead to high surface-area electrodes for the immobilization of binding reagents directly on the conductive particles, especially when the particles are fibers which can extend into the solution.

Detailed Description Text (361):
Modification of a composite surface may shift the electrochemical potential required to excite ECL. Modification of the composite electrode may reduce or increase the overpotential required for excitation of ECL from an ECL tag, thereby allowing certain signals, e.g. the signal from an analyte and a background signal, to be resolved.

Detailed Description Text (362):
The formation of PMAMS on the surface of a composite electrode can be achieved by a variety of methods including photolithographic immobilization, microcontact printing and/or the controlled application of drops of binding reagents to the surface through the use of microcapillary arrays or ink-jet printing (see Sec. 5.1). Alternatively, the surface of a composite electrode may be divided into distinct regions by placing it in contact with a mask.

Detailed Description Text (363):
The invention includes a disposable multiwell plate for use in ECL assays (hereon referred to as an "ECL Plate"). In one embodiment, an ECL Plate is manufactured by shaping (e.g., pressing, molding, or forming) a conductive composite into the form of a multiwell microtiter plate. In another embodiment, a mask is formed that comprises an array of holes through a sheet of a material. Such a mask is then sealed against an electrode (the electrode is preferably a conducting composite or a fibril mat; the preparation of fibril mats is described in detail in Section 5.7, Section 5.18 and the references therein). The holes through the mask will then define wells with walls comprising the mask and bottom comprising the electrode. The mask and the electrode may be provided to the user as a preassembled disposable cassette, or as individual disposable components of a kit. Alternatively, only the electrode may be disposable. The electrode may be solid and/or porous. In the case of a porous electrode, binding reactions may be carried out by filtering reagents through the electrode (multiwell filtration manifolds for use in binding assays--"dot blots"--are known in the art). In a different embodiment of the ECL Plate, a plurality of holes in a mask (as described in the previous embodiment) is sealed against a plurality of individual electrodes such that the electrodes in individual wells and/or groups of wells can be individually probed.

Detailed Description Text (364):
An ECL plate is preferable shaped in a standard form used for multiwell microtiter plates. These standard formats are known in the art and include, but are not limited to, 24, 96, and 384 well plates. The use of a standard format allows the integration of commercially available equipment for carrying out binding reactions on microtiter plates (e.g., equipment for moving plates, washing plates and/or dispensing samples). The invention includes an apparatus for exciting ECL from the electrode or electrodes of an ECL Plate and quantifying the ECL emitted from each well.

Detailed Description Text (366):
Composite electrodes may be used in assays that do not use ECL. They may be used as solid-phase binding supports for assays based on fluorescence, chemiluminescence, or ELISA-type formats. They may be used as electrodes and/or solid phase supports

for assays based on amperometric or potentiometric electrochemical detection.

Detailed Description Text (367):
5.18. ECL Assays Employing PMAMS on a Porous Electrode

Detailed Description Text (368):
The electrode of the invention may comprise a mat of a multiplicity of carbon fibrils. Such mats have now been found to perform well as electrodes for use in electrochemiluminescence assays.

Detailed Description Text (369):
The mats broadly comprise a multiplicity of carbon fibrils and at least one domain containing an assay reagent. In one embodiment of the invention the mat may be comprised of two or more layers of different conductivity, two or more layers of derivitized or underivitized carbon fibrils or combinations of derivitized and underivitized fibrils, two or more layers of fibrils of different optical opacity or two or more layers of fibrils of different pore sizes.

Detailed Description Text (370):
Desirably these mats are used in electrodes for electrochemiluminescence assays. The electrode includes a support and a fibril mat comprising a multiplicity of carbon fibrils and means for making electrical contact with the mat. The electrode may contain a binding domain containing a reagent capable of binding a component of an electrochemiluminescence assay.

Detailed Description Text (371):
The invention includes kits for making electrodes for use in such assays. The kits include a support, a fibril mat and means for making electrical contact with the mat. The fibril mat may include a binding domain.

Detailed Description Text (372):
The electrode may be conductive or porous and desirably is conductive and porous and may be, for example, comprised of a metal-coated porous material. The electrode may be stainless steel fiber mesh.

Detailed Description Text (373):
Fibril mats for use as a support for an electrode in an electrochemiluminescence assay may be prepared in several different ways. In one such method the fibrils are produced with a binding reagent immobilized on their surface. These fibrils are dispersed in a medium. They are thereafter filtered from solution to produce a fibril mat.

Detailed Description Text (375):
The invention broadly includes methods for performing an electrochemiluminescence binding assay for an analyte of interest. The method includes the steps of (a) an electrode comprised of a conductive polymer; and (b) a binding domain containing a reagent capable of binding a component of a binding electrochemiluminescence assay.

Detailed Description Text (376):
The method of the invention can be used to conduct electrochemiluminescence binding assays for a plurality of analytes of interest in a biological sample. This method includes the steps of (a) contacting a sample containing analyte of interest and a label compound capable of electrochemiluminescence, with an electrode comprising a multiplicity of carbon fibrils containing a binding domain containing a reagent capable of binding a component of an electrochemiluminescence assay; (b) inducing the label compound at the electrode to luminesce by imposing a voltage thereupon; and (c) detecting the emitted luminescence.

Detailed Description Text (377):

Alternatively, the method includes (a) contacting a sample containing a plurality of analytes of interest and a label compound capable of electrochemiluminescence with a plurality of electrode zones, each of which comprises a fibril mat containing a domain containing a reagent capable of binding a component of an electrochemiluminescence assay; (b) inducing the label compound collected on said fibril mats to electrochemiluminescence; and (c) measuring the emitted luminescence.

Detailed Description Text (378):
The invention also includes a cassette containing a porous electrode. The cassette contains a working electrode consisting of a porous mat of carbon fibrils supported by a porous material. One or more binding domains are present on the surface of the working electrode.

Detailed Description Text (379):
The porous electrode may be comprised of carbon e.g., graphitic carbon, glassy carbon or carbon fibers and in a particularly preferred embodiment comprises carbon fibrils. The binding domains of the PMAMS may be supported by a fibril mat (see Sec. 5.7). The mats may support a plurality of discrete binding domains, any two or more of which may be identical to each other or all of which may differ from one another. The fibril mat may, alternatively, support one binding domain.

Detailed Description Text (388):
Where filter membranes do not capture fibrils efficiently by filtration, methods can be used to improve filtration efficiency. The effective pore size of the membrane can be reduced by deposition of metals on the surface and/or interior regions of the filter. The filter membrane can be partially plugged or occluded with a material of appropriate size, i.e. a filter aid can be used. The filter can be treated chemically to induce binding between the fibrils and the filter. Binding may be by means of covalent bonds, van der Waals forces, hydrogen bonding, charge/charge interactions, or by hydrophobic hydrophilic interactions, or by biospecific bonding (protein/ligand, antibody/antigen, etc.). The fibrils may be captured by other mechanisms e.g. deposition on the surface of the filter by evaporation of the liquid in which they are suspended.

Detailed Description Text (389):
A filter that supports a fibril mat can work as an electrode for ECL. Examples include filters composed of, or coated with, gold, platinum, carbon, and/or indium-tin oxide(ITO). In such embodiments, both the support and the fibril mat may contribute to the observed ECL signal. In some embodiments, a filter that supports a fibril mat does not function as an electrode for ECL. Such filters provide support and electrical connectivity for the fibril mat, but do not contribute to the observed ECL signal including the background ECL signal.

Detailed Description Text (390):
Fibril mats can also be supported on non-porous materials. Fibril mats may be supported on a material capable of acting as an ECL electrode such as gold foil, platinum foil, conducting composites or ITO. Fibril mats may be supported on a material that cannot function as an ECL electrode such as stainless steel, nickel or non-conducting materials.

Detailed Description Text (392):
The types of assays that can be conducted using PMAMS immobilized on fibril mats include those described in section 5.10. Because the fibril mat is porous, it is possible to conduct assays by flowing the reagents through the fibril mat and in some cases the underlying support. Because the size of the pores in a fibril mat may be small (for example, 10-10000 nm), flowing the reagents through the mat mixes the reagents efficiently. This reduces the time required to conduct an immunoassay by improving the rate of mass transfer to the surface of the binding regions. Assays conducted by wicking a sample into or through the mat benefit similarly from

increased kinetics. Alternatively, the fibril mat may be soaked in the sample. The fibril mat can also act as a filter to remove unwanted materials from biological samples.

Detailed Description Text (393):
Fibril mat electrodes may be used in assays that do not use ECL. They may be used as solid-phase binding supports for assays based on fluorescence, chemiluminescence, or ELISA-type formats. They may be used as electrodes and/or solid phase supports for assays based on amperometric or potentiometric electrochemical detection.

Detailed Description Text (395):
It has also now been discovered that two or more signals originating from electrochemiluminescence species in an electrochemiluminescence assay can be resolved by conducting the assay at an electrode having at least two zones which have different electrochemical potentials at which electrochemiluminescence occurs. By this method it is possible to resolve signal from background electrochemiluminescence and thereby significantly improve the performance of the assay.

Detailed Description Text (397):
Another method for resolving two or more signals from electrochemiluminescence species comprises conducting the assay and an electrode which includes one zone which is inactive for generating electrochemiluminescence from one or more of the species in the assay.

Detailed Description Text (398):
Background signals can be distinguished from a desired signal in an assay by conducting the assay at an electrode which induces electrochemiluminescence for the label and for the background, respectively, at different electrochemical potentials. Likewise, the signals from two or more species labeled with the same electrochemiluminescent compound can be distinguished from one another at an electrode which induces the electrochemiluminescence from each of the labels at different potentials. These improved methods can be carried out on composite electrodes, desirably those comprised of carbon and best results are obtained by those which have been modified by chemical or physical treatment to change the electrochemical potential at which electrochemiluminescence takes place.

Detailed Description Text (399):
The invention includes methods for performing an electrochemiluminescence binding assay for an analyte of interest which comprises the steps of (a) contacting a sample containing analyte of interest and a label compound capable of electrochemiluminescence, with an electrode comprising a multiplicity of carbon fibrils containing a binding domain containing a reagent capable of binding a component of the assay, the carbon fibrils having been modified by chemical or physical treatment to alter the electrochemical potential at which electrochemiluminescence of at least one species in the assay occurs; (b) inducing the label compound at said electrode to luminesce by imposing a voltage thereupon; and (c) detecting the emitted luminescence.

Detailed Description Text (406):
The selective shift in electrochemical potential that is illustrated in FIG. 58 can be accomplished by choosing a material for a working electrode according to the electrochemical potentials at which ECL is elicited from one or more labels in proximity to the electrode. Alternatively, a material may be modified by chemical or mechanical treatment so as to change the electrochemical potential of the ECL signal for one or more components of a sample.

Detailed Description Text (407):
The electrode may have two or more regions with different electrochemical

properties so that one region of the electrode excites an ECL label at a different electrochemical potential than another region. The electrode may be a two-layered fibril in which layer 1 has been derivatized with a binding reagent that binds one or more analytes from a sample and molecules bearing ECL labels and layer 2, conversely, has not been derivatized. It does not bind the analytes but can interact with other components of the sample that give a background ECL signal. As a consequence of the derivatization, layer 1 has different electrochemical properties than layer 2. The ECL signal from labeled molecules bound to layer 1 appears at a higher electrochemical potential than the background ECL signals that originate from layer 2.

Detailed Description Text (410):
The selective change in the intensities of ECL signals illustrated in FIG. 59 can be accomplished by adding a material that quenches the ECL signal for one or more components of a sample. Alternatively, The working electrode may have two or more regions with different electrochemical properties, e.g., an electrode may have one or more regions (R1) that can trigger ECL ("ECL-active") and one or more regions (R2) that cannot trigger ECL ("ECL-inactive"). Components (A1) of a sample bound to regions R1 give an ECL signal in the presence of an appropriate electrochemical potential while the components (B2) of a sample bound to R2 give no ECL signal.

Detailed Description Text (411):
The term "ECL-inactive" can also describe regions of an electrode that produce a non-zero ECL signal that is substantially smaller than the ECL signal from other regions of an electrode or a different electrode. A given material may be ECL-active under some conditions, e.g. in the presence of buffers or certain ECL labels and be ECL-inactive under different conditions.

Detailed Description Text (412):
An electrode can be composed of fibrils, which are ECL active, and a support which is ECL inactive. In this embodiment, the components of the sample that are in electrochemical contact with the fibrils emit an ECL signal when the proper electrochemical potential is applied. In contrast, components of the sample that are in electrochemical contact with the ECL-inactive support and not the fibrils do not give an ECL signal when the electrochemical potential is applied.

Detailed Description Text (413):
The optical opacity of an electrode can be used to selectively prevent detection of ECL signals from one or more components of a sample (see Sec. 5.11 and FIG. 29).

Detailed Description Text (414):
An electrode may be ECL active for one or more components of a sample and ECL inactive for other components.

Detailed Description Text (415):
In another embodiment, one or more components (A.sub.n) of a sample can be in electrochemical contact with an ECL active electrode and one or more components (B.sub.n) of a sample can be out of electrochemical contact with an ECL electrode, i.e. they are not in sufficient proximity to the electrode. When an appropriate electrochemical potential is applied to the electrode, an ECL signal originates from components A.sub.n and not from components B.sub.n. An electrode may consist of a porous, ECL active layer bearing one or more binding domains for analytes A.sub.n and a porous, ECL inactive layer. When a sample is filtered through this electrode, some analytes A.sub.n bind to the ECL active layer, and unbound components are captured in the ECL-inactive layer. When a potential is applied to the electrode, ECL is triggered for the bound components A, since they are bound to an ECL active layer, but not for the other components, since they are entrained in an ECL inactive layer. The invention is further described in the following examples which are in no way intended to limit the scope of the invention.

Detailed Description Text (420):
It has been found that ECL sandwich immunoassays using capture antibodies located on a working electrode, the binding reaction can take more than 1/2 hour to reach completion, even when vortexing is used to increase mass transport to the solid-support surface. This time scale is also typical of other highly sensitive solid-phase binding assays, such as ELISA and RIA. Unexpectedly, we found that sonication of reagents reduced the time required for completion of these binding reactions to a matter of minutes. The apparatus and methodology of the invention is not limited to immunoassays and will be useful for a wide variety of binding interactions (e.g., nucleic acid hybridization, antigen-antibody, receptor-ligand, enzyme-substrate, etc.).

Detailed Description Text (431):
The present invention is generally applicable to binding assay systems such as immunoassays, nucleic acid hybridization assays, receptor-ligand binding assays, and the like. In assays where binding reactions occur in the vicinity of an electrode, sonication of the electrode itself has proven to have an especially beneficial effect in increasing assay reaction rates.

Detailed Description Text (437):
An assay system 690100 for conducting ECL assays in a disposable cartridge 69090 with an instrument 690101 is illustrated in FIG. 69. Cartridge 69090 includes a base 69091, a diaphragm 69092, a counterelectrode 69093, a reaction enclosure 69094, a sample port 69095, electrical leads 69096, and a reference electrode 69099. Instrument 690100 includes a cartridge receptacle 690108, a light detector and/or imaging device 690102, an electrical connector 690103, a source of electrical energy for applying a voltage or current between the working and counter electrodes 690104; a sonication device 690105; a source of electrical energy 690106 for driving sonication device 690105; and a microprocessor 690107 for instrument control, assay data gathering, and assay data analysis.

Detailed Description Text (438):
Diaphragm 69092 is an electrically conductive solid-phase support for reagents 69097A, such as binding reagents, and functions as a working electrode. In a preferred embodiment, diaphragm 69092 is a fibril-polymer composite electrode and reagents 69097A comprise binding reagents such as antibodies, nucleic acids, receptors, etc. immobilized thereon. In an especially preferred embodiment, binding reagents specific for a variety of analytes are patterned into binding domains on diaphragm 69092. Base 69091 is preferably a rigid and transparent material, such as acrylic or the like, that allows light generated by an ECL reaction occurring within enclosure 69094 to be detected by detector 690102. Base 69091 is shaped to define reaction enclosure 69094 and sample port 69095. Diaphragm 69092 is preferably sealed to base 69091.

Detailed Description Text (439):
Electrical leads 69096 are electrical contacts providing electrical coupling to diaphragm 69092 and to counter electrode 69093. Preferably, diaphragm 69092 is mounted such that the transmission of sonication energy from device 690105 to base 69091 is minimized. Alternatively, diaphragm 69092 may be mounted so that diaphragm 69092 transmits sonication energy from device 690105 to base 69091, and thereon to the entire surface of reaction enclosure 69094.

Detailed Description Text (441):
Counter electrode 69093 is preferably an electrically conductive material, such as metal. Reference electrode 69099 is preferably an Ag/AgCl reference electrode. Electrodes 69093 and 69099 are disposed within base 69091, are coupled to leads 69096, and are adapted to be in electrical contact with reagents 69098. Optionally, reference electrode 69098 may be omitted. Aperture 69095 is preferably adapted for insertion of sample material (e.g., reagents 69098) via a small tube (not shown), such as a capillary tube.

Detailed Description Text (442):
The inner surface of instrument 690101 is adapted to receive and align cartridge 69090 and its components with receptacle 690108 and its counterpart components, including sonication device 690105, electrical connections 690103 and detector 690102. Preferably, detector 690102 is an array of detectors (e.g., a CCD camera or a photodiode array) that can image the light emitted during an ECL reaction at the working electrode. Detector 690102 may be a single detector such as a photomultiplier tube, a photodiode, or the like. Insertion of cartridge 69090 in instrument 690101 aligns detector 690102 with base 69091 such that detector 690102 is positioned to detect much of the light produced within enclosure 69094.

Detailed Description Text (443):
Sonication device 690105 is a device for sonicating diaphragm 69092 which transmits the sonication energy to reagents 69098 contained in reaction enclosure 69094. Insertion of cartridge 69090 in instrument 690101 preferably aligns device 690105 with the center of diaphragm 69092 such that device 690105 may be moved into contact with diaphragm 69092. Insertion of cartridge 69090 in instrument 690101 causes sonication device 690105 to be structurally coupled to electrode 69092. It is preferred that sonication device 690105 comprises a piezoelectric sonication device that may include a piston. Preferably, sonication device 690105 is movable to achieve contact with diaphragm 69092 when cartridge 69090 is inserted into instrument 690101.

Detailed Description Text (444):
Upon insertion of cartridge 69090 into receptacle 690108, electrical leads 69096 are coupled to electrical connections 690103. The source of electrical energy 690104 may be a controllable voltage or current source adapted for control by microprocessor 690107. Alternatively, if cartridge 69090 includes a reference electrode, source 690104 is preferably a potentiostat.

Detailed Description Text (448):
The sonication causes reagents 69098 and reagents 69097B to mix, speeding the rate of reaction among components reagents 69098 and/or 69097B and the rate of mass transfer of reagents 69098 and/or 69097B to and from diaphragm 69092. Sonication energy from device 690105 significantly increases the rate of mass transfer of reagents 69098 and/or 69097B to support 69092, thereby increasing the rate of binding reactions between reagents 69097A and components of reagents 69097B and 69098, and decreasing the time required to make an ECL measurement. Electrical energy is applied to diaphragm 69092 and to electrodes 69093, by source 690104 via connector 690103 and leads 690106, to cause an electrochemiluminescent moiety in reactants 69097A, 69097B and/or 69098 to luminesce. The light produced by the ECL reaction may be measured (or imaged) while sonication device 690105 operates or thereafter.

Detailed Description Text (451):
In an alternate embodiment, diaphragm 69092 and/or enclosure 69094 are pre-coated with a reagent or the like. Sonication of electrode 69092 may cause such reagent to loosen, allowing the reagent to mix with reagents 69098 within enclosure 69094.

Detailed Description Text (453):
The interior surfaces of reaction enclosure 69094 may become coated with a substance that interferes with an assay. This interfering substance may include a contaminant, cellular debris, a non-specifically bound reagent, a reaction byproduct, or the like. In yet another embodiment of the invention, sonication device 690105 is activated and the sonication energy removes the interfering substances from the interior surfaces of enclosure 69094 by sonicating such substances to loosen or by causing increasing the rate of mass transport at the surfaces. For example, an ECL assay may use cleaning cycles involving activation of device 690105 before and/or after the binding reaction to properly prepare the

electrode for the excitation of ECL. These cleaning cycles may involve adding to reaction enclosure 69094 a cleaning solution which assists in loosening such interfering substances.

Detailed Description Text (465):
A transparent PMAMS surface is made as described above which is substantially transparent with a patterned multi-specific array of primary antibodies linked to the surface. The support, electrode assay, monologues surface use selected to be transparent. The PMAMS surface is then exposed to a solution sample suspected of containing an analyte of interest to be assayed. The sample is then washed away leaving antibody bound analytes on the surface. The PMAMS surface is then exposed to a solution containing secondary ECL-labeled antibodies specific for bound analytes on the surface. This solution is then washed from the PMAMS surface leaving ECL labeled secondary antibodies bound to the domains where analyte is present.

Detailed Description Text (466):
The electrode assay is protected by a removable barrier to prevent premature contact of the sample with the electrode surface in order to avoid contamination effects. The barrier is then removed and the electrode array, that is wetted with assay buffer, is brought into aligned contact with the PMAMS surface. The electrode array is connected to an electronic potential wave form generator, and potential is applied to working electrode/counterelectrode pairs. A CCD then reads the light emitted and the signal is sent to a microprocessor which converts the signal to the desired readout form.

Detailed Description Text (471):
The barrier protecting the second PMAMS in register with the first PMAMS is removed and the micro-drops are brought into register with the primary antibody binding domains on the first PMAMS. The second PMAMS is lifted off and the electrode array and is brought into aligned contact with the first PMAMS surface. The electrode array is connected to an electrical potential wave form generator, and potential is applied to working electrode/counterelectrode pairs. A photo multiplier tube then reads the light emitted and the signal is sent to a microprocessor which converts the signal to the desired readout form.

Detailed Description Text (475):
The barrier protecting the second PMAMS in register with the first PMAMS is removed and the micro-drops are brought into register with the primary antibody binding domains on the first PMAMS. The second PMAMS is lifted off and the electrode array and is brought into aligned contact with the first PMAMS surface.

Detailed Description Text (476):
The electrode array is connected to an electronic potential wave form generator, and potential is applied to working electrode/counterelectrode pairs. A CCD then reads the light emitted and the signal is sent to a microprocessor which converts the signal to the desired readout form.

Detailed Description Text (480):
The electrode array is protected by a removable barrier to prevent contact of the sample with the electrode surface in order to avoid contamination effects. The barrier is then removed and the electrode array, that is wetted with assay buffer, is brought into aligned contact with the PMAMS surface. The electrode array is connected to a electronic potential wave form generator, and potential is applied to working electrode/counterelectrode pairs. A photomultiplier tube then reads the light emitted and the signal is sent to a microprocessor which converts the signal to the desired readout form.

Detailed Description Text (485):
The barrier protecting the second PMAMS in register with the first PMAMS is removed

and the micro-drops are brought into register with the primary antibody binding domains on the first PMAMS. The second PMAMS is lifted off and the electrode array is brought into aligned contact with the PMAMS surface. The electrode array is connected to a electronic potential wave form generator, and potential is applied to working electrode/counterelectrode pairs. A CCD then reads the light emitted and the signal is sent to a microprocessor which converts the signal to the desired readout form.

Detailed Description Text (494):
An electrode array of interdigitating working and counterelectrode pairs on a gold on silicon surface is fabricated through methods known in the art (for example see Kumar et al supra). In this example, the electrode array and the discrete binding domain array exist on the same surface of a support. An exposed and developed photoresist master of 1-2 microns thickness is prepared according to well known procedures in the pattern of the working electrodes. A 10:1 mixture of SYLGARD silicone elastomer 184 (poly(dimethylsiloxane(PDMS)); available from Dow Corning) and the corresponding SYLGARD 184 curing agent is poured over the master and cured. The polymerized SYLGARD 184 is carefully removed from the silicon master. The resulting elastomeric stamp is "inked" by exposure to a hydrophilic OH-terminated alkane thiol, SH(CH.sub.2).sub.11 --(OCH.sub.2 CH.sub.2).sub.6 OH, in an ethanolic solution (1-10 mM), robotically brought into pin registered contact with the aligned working electrodes on gold electrode array surface, and is then removed. A capillary array containing hydrophilic solutions is then robotically brought into pin registered contact by aligning the capillaries with the SH(CH.sub.2).sub.11 --(OCH.sub.2 CH.sub.2).sub.6 OH domains on the electrode array surface to place specific antibodies on each domain. Each capillary in the capillary array contains monoclonal antibodies, specific for an analyte of interest, capable of covalently binding to the reactive OH groups on the hydrophilic domains through an amide linkage.

Detailed Description Text (496):
A support as described 6.12, supra, is fabricated. A PDMS stamp is fabricated as previously described from a photoresist master patterned as rings which each independently circumscribe a working electrode/counterelectrode pair. The electrode array surface is then exposed to a sample to be analyzed, washed with a mixture of ECL labelled secondary antibodies, and then washed with an assay buffer solution containing tripropyl amine. The PDMS stamp is then aligned and brought into registered contact aligning the rings of the PDMS stamp so as to circumscribe and define individual volume elements of assay buffer above each electrode pair. An overpotential is applied to the electrode pairs so as to release the monolayer from the surface exposing the working electrode to the ECL labelled secondary antibodies. A photomultiplier tube then reads the light emitted through the transparent PDMS and the signal is sent to a microprocessor which converts the signal to the desired readout form.

Detailed Description Text (499):
An electrode array of interdigitating working and counter gold electrode pairs with gold binding domains in between the interdigitating electrodes on a gold on silicon support is fabricated through methods known in the art (for example see Kumar et al. supra). In this example, the electrode array and the discrete binding domain array exist on the same surface. An exposed and developed photoresist master of 1-2 microns thickness is prepared according to well known procedures in the pattern of the binding domains in between the interdigitating electrode pairs. A 10:1 mixture of SYLGARD silicone elastomer 184 (poly(dimethylsiloxane(PDMS)); available from Dow Corning) and the corresponding SYLGARD 184 curing agent is poured over the master and cured. The polymerized SYLGARD 184 is carefully removed from the silicon master. The resulting elastomeric stamp is "inked" by exposure to a hydrophilic OH-terminated alkane thiol, SH(CH.sub.2).sub.11 --(OCH.sub.2 CH.sub.2).sub.6 OH, in an ethanolic solution (1-10 mM), robotically brought into pin registered contact with the aligned gold binding domains on the electrode array surface, and is then

removed. A capillary array containing hydrophilic solutions is then robotically brought into pin registered contact, aligning the capillaries with the SH (CH.sub.2).sub.11 --(OCH.sub.2 CH.sub.2).sub.6 OH domains on the electrode array surface to place specific antibodies on each domain. Each capillary in the capillary array contains antibodies specific for an analyte of interest, capable of covalently binding to the reactive OH groups on the hydrophilic domains through an amide linkage.

Detailed Description Text (501):
A support surface as described 6.14 supra is fabricated by the described methods. The prepared surface is exposed to a sample to be analyzed, washed with a mixture of ECL labelled secondary antibodies, and then washed with an assay buffer solution containing tripropyl amine. The electrode array is connected to a electronic potential wave form generator, and potential is applied to working electrode/counterelectrode pairs. A photomultiplier tube then reads the light emitted and the signal is sent to a microprocessor which converts the signal to the desired readout form.

Detailed Description Text (506):
The support surface described above in example 6.16 is exposed to a sample solution to be analyzed. The support surface is then washed and exposed to a solution containing a plurality of ECL labelled monoclonal antibodies or ECL labelled nucleic acids of differing specificity and then washed with assay buffer containing tripropyl amine. A transparent addressable working electrode array is fabricated with each working electrode in the array corresponding to a discrete binding domain/counterelectrode region on the support as described above in Section 6.16. The two supports are wetted with the assay buffer and robotically brought into registered aligned conformal contact. The electrode arrays are connected to an electronic potential wave form generator, and potential is applied to the aligned working electrode/counterelectrode pairs creating a potential field between the two supports. A CCD then reads the light emitted through the transparent working electrode and the signal is sent to a microprocessor which converts the signal to the desired readout form.

Detailed Description Text (510):
A nylon filter membrane (0.47 .mu.m pore size, 25 mm diameter) was placed in a 25 mm diameter glass fritted filter. The dispersed fibril slurry was filtered through the membrane/filter set-up by suction filtration (FIG. 23A). Aliquots of the slurry (5 ml) were diluted with 20 ml deionized water, then filtered through the membrane/filter set-up. For an average mat of approximately 0.25-0.3 gram/cc, a mat of approximately 100 .mu.m required 6 aliquots.

Detailed Description Text (530):
6.22. Cyclic Voltammograms of Fibril Mats: Comparison of Fibril Mat with Gold Foil Electrode

Detailed Description Text (531):
Cyclic voltammograms of 6 mM Fe.sup.3+ /.sup.2+ (CN).sub.6 in 0.5 M K.sub.2 SO.sub.4 were measured. In FIG. 30A, the CV for a plain fibril mat of CC(dispersed) was measured at 0.10 mA/cm at 10, 25 and 50 mV/sec. The mat was fabricated as described in Example 6.18. In FIG. 30B, the CV was measured for a gold foil electrode at 0.05 mA/cm at 10, 25 and 50 mV/sec. All potentials are in Volts vs. Ag/AgCl.

Detailed Description Text (532):
6.23. Electrochemical Properties of Fibril Mat Electrodes: Comparison of Anodic Peak Current with Thickness of the Mat

Detailed Description Text (533):
Cyclic voltammograms of 6 mM Fe.sup.3+ /.sup.2+ (CN).sub.6 in 0.5 M K.sub.2

SO.sub.4 were measured for fibril mats of the same geometric area (0.20 cm.sup.2), but different thicknesses. The anodic peak current (FIG. 31) increased with increasing thickness of mat for thicknesses that ranged from 24 .mu.m to 425 .mu.m. For each thickness, the anodic peak current also increased with increasing scan rates (for rates that ranged from 10 mV/sec to 150 mV/sec). The rate of increase of the anodic peak current, as a function of thickness, also increased with increasing thickness. Fibril mats that were 24 .mu.m thick behaved comparably to gold foil electrodes.

Detailed Description Text (537):
6.25. Reduction of Non-Specific Binding of Proteins to Fibrils with Detergents/Surfactants

Detailed Description Text (538):
Using the method described in Example 6.2.4, the effect of surfactant on protein binding to fibrils was analyzed. Triton X-100 was added to the anti-CEA attached to derivatized TAG1/fibril mixture, the solution was incubated for 20 minutes, the tubes were centrifuged, and aliquots of the supernatant, diluted 5 times with ORIGEN assay buffer, were analyzed by ECL. The results are shown in the Table below and in FIG. 33.

Detailed Description Text (540):
6.26. ECL of Free TAG in Solution with Fibril Mat Electrode

Detailed Description Text (541):
A fibril mat prepared as in Example 6.18 was installed in the mounting area 3403 of the working electrode holder 3401 of the "Fibril Cell" fixture shown in FIG. 34. The holder 3401 was slipped into the bottom of the electrochemical cell compartment 3400. The 3 M Ag/AgCl reference electrode (Cyprus #EE008) was installed into the electrochemical cell compartment through the reference cell hole 3402. The cell was filled with Assay Buffer (IGEN #402-005-01 lot #5298) and attached to the PMT holder 3404. Using a EG&G PARC model 175 universal programmer and an EG&G model 175 Potentiostat/Galvanostat the potential was swept from 0 V to +3 V vs. Ag/AgCl at 100 mV/s. The ECL was measured by a Hamamatsu R5600U-01 which was powered at 900V by a Pacific Instruments model 126 Photometer. The analog data was digitized at 10 Hz by a CIO-DAS-1601 A/D board driven by HEM Snap-Master. The Fibril Cell was drained, flushed with 1000 pM TAG1 (IGEN #402-004-C lot#4297), and filled with 1000 pM TAG1. The potential was swept as with Assay Buffer. Shown in FIG. 35 are the ECL traces (measured at 24.0.+-.0.2 C) for Assay Buffer 3501 and 1000 pM TAG1 3502. The dark corrected ECL peak area was 22.10 nAs for Assay Buffer and 46.40 nAs for 1000 pM TAG1.

Detailed Description Text (542):
6.27. ECL of Adsorbed Labeled Antibody with Fibril Mat Electrode

Detailed Description Text (543):
Fibril mats were made to a thickness of 0.0035 inches from plain cc-dispersed fibrils in the manner described in Example 6.18. The dried mats were then punched into 3 mm disks and mounted onto supports. The supports used in this experiment were fabricated from 0.030 inches polyester sheet patterned by screen printed conductive gold ink. This conductive gold ink formed the counter electrode, reference electrode, and provided leads for the working and other electrodes. Two fibril mat disks were mounted to each patterned support using two sided carbon containing conductive tape (Adhesives Research). After mounting, the disks were spotted with 0.5 .mu.l of 10 .mu.g/ml anti-TSH antibody attached to derivatized TAG1 in deionized water (Ru-TSH mono 1:2 Jun. 26, 1995, IGEN, Inc.) or 0.5 .mu.l of 10 .mu.g/ml anti-TSH unTAG1'ed capture antibody in deionized water (TSH poly Jun. 25, 1995, IGEN, Inc.) and allowed to dry. After drying, the mats were flooded with IGEN assay buffer. Flooded mats on supports were loaded into an IGEN Origen 1.5 based instrument and ECL was read-using a scan rate of 500 mV/s from 0 to 4500 mV.

FIG. 43 compares the peak ECL signals from TAG1-antibody containing mats 4301 and unTAG1'ed capture antibody containing mats 4302.

Detailed Description Text (544):
6.28. ECL Using Fibril Mat Electrode for Sandwich Assay

Detailed Description Text (545):
Anti-AFP capture antibody was immobilized on fibrils as described above. Anti-AFP fibrils were washed into deionized water (dI) and resuspended at a density of 1 mg/ml. A four layer fibril mat was produced using vacuum filtration as described in Example 6.18. Two milligrams of anti-AFP fibrils were added to 3 mg of plain CC dispersed fibrils and the mixture diluted to a total volume of 20 ml in dI. The diluted mixture was filtered onto a 0.45 .mu.m nylon filter. This initial mat layer was then followed by two core layers, each consisting of 5 mg of plain CC dispersed fibrils. The mat core was then topped with a mixed fibril layer identical to the initial layer. This resulted in a fibril mat that was .about.40% anti-AFP fibrils on the top and bottom surface and .about.100% plain fibrils in the core. This mixed mat was air dried under vacuum and punched into 3 mm disks. These disks were then mounted onto supports as described in Example 6.27. Dry, supported, anti-AFP mats were flooded with AFP calibrators A, C, and F (IGEN, Inc.) and allowed to incubate for 15 minutes at room temperature on the bench top. After incubation, supported electrodes were washed with a dI stream for 10 seconds and then blotted dry with a lint free wipe. Fibril mats were then flooded with anti-AFP attached to antibody labelled with derivatized TAG1 (IGEN, Inc.) and allowed to incubate for 15 minutes at room temperature on the bench top. After incubation the supported electrodes were washed with dI and dried with a wipe. Fibril mats were then flooded with IGEN assay buffer and read as described in Example 6.27.

Detailed Description Text (547):
A cross-linked polyacrylamide gel containing covalently bound biotin was prepared by copolymerization of acrylamide, bis-acrylamide, and N-Acryloyl-N'-biotinyl-3,6-dioxaoctane-1,9-diamine (biotin linked to an acrylamide moiety through a tri (ethylene glycol) linker) using well known conditions (initiation with ammonium persulfate and TEMED). In this experiment, the concentrations of the three monomeric species were 2.6 M, 0.065 M, and 0.023 M respectively (these concentrations of acrylamide and bis-acrylamide are reported to result in gels with pore sizes smaller than most proteins). Polymerization of the solution containing the monomers between two glass plates held apart to a distance of approximately 0.7 mm led to the formation of a slab gel with the same thickness. After the polymerization reaction was complete, any unincorporated biotin was washed out by soaking the gel in four changes of PBS. Avidin labeled with a derivatized TAG1 (where Avidin refers to NeutrAvidin, a modified avidin designed to exhibit reduced NSB, was used in this experiment) was bound to the surface of the gel by soaking the gel in a solution containing the protein at a concentration of 50 .mu.g/mL in PBS for 20 min. Excess TAG1-labeled avidin was then washed away by soaking the gel in four changes of ECL assay buffer (200 mM sodium phosphate, 100 mM tripropylamine, 0.02% (w/v) Tween-20, pH 7.2). As shown in FIG. 39, the gel (3900) was then placed in contact with gold working (3901) and counter (3902) electrodes patterned on a glass support (3903). Ramping the potential across the two electrodes from 0.0 to 3.0 V and back to 0.0 V at a rate of 500 mV/s led to an ECL light signal as measured from a PMT (3904) placed above the gel (FIG. 40). A gel prepared without inclusion of the biotin containing acrylamide derivative gave no ECL signal (FIG. 41). This signal obtained for the biotin-containing polymer was indicative of close to a full monolayer of protein is present on the surface of the gel.

Detailed Description Text (550):
6.31. Multiple ECL Sandwich Immunoassays on Polyacrylamide Surfaces Supported on an Electrode

Detailed Description Text (551):
An exposed and developed photoresist master of 1-2 microns thickness is prepared according to well known procedures to give a pattern of circular depressions arranged in an array. A 10:1 mixture of SYLGARD silicone elastomer 184 and the corresponding SYLGARD 184 curing agent is poured over the master and cured. The polymerized SYLGARD is carefully removed from the silicon master. The resulting elastomeric stamp is "inked" by exposure to a solution containing the hydroxyl terminated thiol HS--(CH.sub.2).sub.11 --(OCH.sub.2 CH.sub.2).sub.3 --OH (1-10 mM) in ethanol, brought into contact with an aligned gold substrate and removed. The substrate is washed for several seconds with a solution containing the thiol HS--(CH.sub.2).sub.10 --CH.sub.3 (1-10 mM in ethanol). The resulting surface is then rinsed with ethanol and dried under a stream of nitrogen. Treatment of the surface with a solution containing acryloyl chloride and triethylamine in dioxane leads to functionalization of the hydroxyl terminated domains with acrylate groups. A capillary array containing mixtures of acrylamide, bis-acrylamide, N-acryloylsuccinimide, azo-bis-cyanovaleric acid, and antibodies presenting amino groups is then brought into contact with the aligned surface aligning the capillaries with the acrylate terminated domains to place prepolymer solutions containing specific antibodies at each domain. Each capillary in the capillary array contains antibodies specific for a different analyte of interest. Exposure of the patterned prepolymer droplets to UV light leads to the formation of cross-linked gels on the substrate each presenting a binding domain at the surface. The assay is carried out by treatment of the substrate with a mixture of analytes capable of binding at one or more of the binding domains presented on the gel surfaces in a buffered solution containing tripropylamine and ECL-TAG1 labeled secondary antibodies. The binding domains (4200, 4201, 4202) (on polyacrylamide drops (4203) on a gold electrode (4232) are then placed in close proximity to an ITO working electrode (4204) as shown in FIGS. 42A-B. Light emitted from each of the binding domains is quantified using a CCD camera (4205) and compared to binding domains for internal standards included in the sample solution.

Detailed Description Text (552):
6.32. Multiple ECL Competitive Immunoassays on Polyacrylamide Surfaces Supported on an Electrode

Detailed Description Text (553):
An exposed and developed photoresist master of 1-2 microns thickness is prepared according to well known procedures to give a pattern of circular depression arranged in an array. A 10:1 mixture of SYLGARD silicone elastomer 184 and the corresponding SYLGARD 184 curing agent is poured over the master and cured. The polymerized SYLGARD is carefully removed from the silicon master. The resulting elastomeric stamp is "inked" by exposure to a solution containing the hydroxyl terminated thiol HS--(CH.sub.2).sub.11 --(OCH.sub.2 CH.sub.2).sub.3 --OH (1-10 mM) in ethanol, brought into contact with an aligned gold substrate and removed. The substrate is washed for several seconds with a solution containing the thiol HS--(CH.sub.2).sub.10 --CH.sub.3 (1-10 mM in ethanol). The resulting surface is then rinsed with ethanol and dried under a stream of nitrogen. Treatment of the surface with a solution containing acryloyl chloride and triethylamine in dioxane leads to functionalization of the hydroxyl terminated domains with acrylate groups. A capillary array containing mixtures of acrylamide, bis-acrylamide, N-acryloylsuccinimide, azo-bis-cyanovaleric acid, and antibodies is then brought into contact with the aligned surface aligning the capillaries with the acrylate terminated domains to place prepolymer solutions containing specific antibodies at each domain. Capillaries in the capillary array contain antibodies specific for different analytes of interest. Exposure of the patterned prepolymer droplets to UV light leads to the formation of cross-linked gels on the substrate each presenting a binding domain at the surface. The assay is carried out by treatment of the substrate with a mixture of analytes capable of binding at one or more of the binding domains presented on the gel surfaces in a buffered solution containing tripropylamine and ECL-TAG1 labeled analogues of the analytes (i.e., setting up a

competition of ECL-TAG1 labeled and unlabeled analytes for binding to the binding domains). The binding domains (4200, 4201 and 4202) (on polyacrylamide drops (4203) on a gold electrode (4232)) are then placed in close proximity to an ITO working electrode (4204) as shown in FIG. 42. Light emitted from each of the binding domains is quantified using a CCD camera (4205) and compared to binding domains for internal standards included in the sample solution.

Detailed Description Text (554):
6.33. Multiple ECL Assays for Binding of Cells on Polyacrylamide Surfaces Supported on an Electrode

Detailed Description Text (555):
An exposed and developed photoresist master of 1-2 microns thickness is prepared according to well known procedures to give a pattern of circular depressions arranged in an array. A 10:1 mixture of SYLGARD silicone elastomer 184 and the corresponding SYLGARD 184 curing agent is poured over the master and cured. The polymerized SYLGARD is carefully removed from the silicon master. The resulting elastomeric stamp is "inked" by exposure to a solution containing the hydroxyl terminated thiol HS--(CH.sub.2).sub.11 --(OCH.sub.2 CH.sub.2).sub.3 --OH (1-10 mM) in ethanol, brought into contact with an aligned gold substrate and removed. The substrate is washed for several seconds with a solution containing the thiol HS--(CH.sub.2).sub.10 --CH.sub.3 (1-10 mM in ethanol). The resulting surface is then rinsed with ethanol and dried under a stream of nitrogen. Treatment of the surface with a solution containing acryloyl chloride and triethylamine in dioxane leads to functionalization of the hydroxyl terminated domains with acrylate groups. A capillary array containing mixtures of acrylamide, bis-acrylamide, N-acryloylsuccinimide, azo-bis-cyanovaleric acid, and antibodies directed against cell surfaces is then brought into contact with the aligned surface aligning the capillaries with the acrylate terminated domains to place prepolymer solutions at each domain. Exposure of the patterned prepolymer droplets to UV light leads to the formation of cross-linked gels on the substrate each presenting a binding domain at the surface. The assay is carried out by treatment of the binding domains first with a suspension of cells, then with a mixture of binding reagents capable of binding one or more of the cells bound to the gel surfaces in a buffered solution containing tripropylamine and ECL-TAG1 labeled secondary antibodies and/or other binding reagents specific for the analytes. The binding domains (4200, 4201, and 4202) (on polyacrylamide drops (4203) on a gold electrode (4232) are then placed in close proximity to an ITO working electrode (4204) as shown in FIG. 42. Light emitted from each of the binding domains is quantified using a CCD camera (4205) and compared to binding domains for internal standards included in the sample solution.

Detailed Description Text (556):
6.34. Multiple ECL Assays for Binding of Analytes to Cells on Polyacrylamide Surfaces Supported on an Electrode

Detailed Description Text (557):
An exposed and developed photoresist master of 1-2 microns thickness is prepared according to well known procedures to give a pattern of circular depressions arranged in an array. A 10:1 mixture of SYLGARD silicone elastomer 184 and the corresponding SYLGARD 184 curing agent is poured over the master and cured. The polymerized SYLGARD is carefully removed from the silicon master. The resulting elastomeric stamp is "inked" by exposure to a solution containing the hydroxyl terminated thiol HS--(CH.sub.2).sub.11 --(OCH.sub.2 CH.sub.2).sub.3 --OH (1-10 mM) in ethanol, brought into contact with an aligned gold substrate and removed. The substrate is washed for several seconds with a solution containing the thiol HS--(CH.sub.2).sub.10 --CH.sub.3 (1-10 mM in ethanol). The resulting surface is then rinsed with ethanol and dried under a stream of nitrogen. Treatment of the surface with a solution containing acryloyl chloride and triethylamine in dioxane leads to functionalization of the hydroxyl terminated domains with acrylate groups. A

capillary array containing mixtures of acrylamide, bis-acrylamide, N-acryloylsuccinimide, azo-bis-cyanovaleric acid, and cells is then brought into contact with the aligned surface aligning the capillaries with the acrylate terminated domains to place prepolymer solutions containing specific cell types at each domain. Capillaries in the capillary array contain cells with different surface structures that bind different analytes. Exposure of the patterned prepolymer droplets to UV light leads to the formation of cross-linked gels on the substrate each presenting a binding domain at the surface. The assay is carried out by treatment of the gels with a sample containing a mixture of analytes capable of binding at one or more of the binding domains presented on the gel surfaces in a buffered solution containing tripropylamine and ECL-TAG labeled antibodies and/or other binding reagents specific for the analytes. The binding domains (4200, 4201 and 4202) (on polyacrylamide drops (4203) on a gold electrode (4232) are then placed in close proximity to an ITO working electrode (4204) as shown in FIG. 42. Light emitted from each of the binding domains is quantified using a CCD camera (4205) and compared to binding domains for internal standards included in the sample solution.

Detailed Description Text (558):
6.35. Multiple ECL Competitive Hybridization Assays on Polyacrylamide Surfaces Supported on an Electrode

Detailed Description Text (559):
An exposed and developed photoresist master of 1-2 microns thickness is prepared according to well known procedures to give a pat

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INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wohlstadter; Jacob N.	Rockville	MD		
Wilbur; James	Germantown	MD		
Sigal; George	Rockville	MD		
Martin; Mark	Rockville	MD		
Guo; Liang-Hong	Gaithersburg	MD		
Fischer; Alan	Cambridge	MA		
Leland; Jon	Silver Spring	MD		
Billadeau; Mark A.	Mt. Airy	MD		
Helms; Larry R.	Germantown	MD		
Darvari; Ramin	Waltham	MA		

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INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hubbell; Jeffrey A.	Austin	TX		
Pathak; Chandrashekhar P.	Waltham	MA		
Sawhney; Amarpreet S.	Newton	MA		
Desai; Neil P.	Los Angeles	CA		
Hill; Jennifer L.	Austin	TX		
Hossainy; Syed F. A.	Austin	TX		

US-CL-CURRENT: 424/423; 424/422, 424/426, 424/484, 424/486, 424/487, 424/488, 424/78.08, 424/78.37, 424/93.1, 424/93.7, 424/94.1, 435/177, 435/178, 435/180, 435/182, 435/382, 435/395, 514/2, 530/812, 530/813, 530/815, 530/817

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. De

☐ 3. Document ID: US 6465001 B1

L16: Entry 3 of 15 File: USPT Oct 15, 2002

US-PAT-NO: 6465001
DOCUMENT-IDENTIFIER: US 6465001 B1

TITLE: Treating medical conditions by polymerizing macromers to form polymeric materials

DATE-ISSUED: October 15, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hubbell; Jeffrey A.	Zumikon			CH
Pathak; Chandrashekhar P.	Austin	TX		
Sawhney; Amarpreet	Bedford	MA		
Desai; Neil	Los Angeles	CA		
Hossainy; Syed	Edison	NJ		
Hill-West; Jennifer L.	Pearland	TX		

US-CL-CURRENT: 424/423; 424/422, 424/426, 424/484, 424/486, 424/487, 424/488, 424/78.08, 424/78.31, 424/93.1, 424/93.7, 424/94.1, 435/178, 435/180, 435/182, 435/197, 435/382, 435/395, 514/2, 530/812, 530/813, 530/815, 530/817

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw. De

☐ 4. Document ID: US 6258870 B1

L16: Entry 4 of 15 File: USPT Jul 10, 2001

US-PAT-NO: 6258870
DOCUMENT-IDENTIFIER: US 6258870 B1

TITLE: Gels for encapsulation of biological materials

h e b b g e e e f e e ef b e

DATE-ISSUED: July 10, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hubbell; Jeffrey A.	Concord	MA		
Pathak; Chandrashekhar P.	Waltham	MA		
Sawhney; Amarpreet S.	Newton	MA		
Desai; Neil P.	Los Angeles	CA		
Hossainy; Syed F. A.	Austin	TX		

US-CL-CURRENT: 522/26; 424/487, 424/489, 424/493, 424/499, 424/93.1, 427/213.3, 427/213.31, 427/213.32, 427/213.35, 435/177, 435/178, 435/182, 522/44, 522/75, 522/84

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

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☐ 5. Document ID: US 6207369 B1

L16: Entry 5 of 15 File: USPT Mar 27, 2001

US-PAT-NO: 6207369
DOCUMENT-IDENTIFIER: US 6207369 B1

TITLE: Multi-array, multi-specific electrochemiluminescence testing

DATE-ISSUED: March 27, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wohlstadter; Jacob N.	Rockville	MD		
Wilbur; James	Rockville	MD		
Sigal; George	Gaithersburg	MD		
Martin; Mark	Rockville	MD		
Guo; Liang-Hong	Laurel	MD		
Fischer; Alan	Cambridge	MA		
Leland; Jon	Silver Spring	MD		
Billadeau; Mark A.	Mt. Airy	MD		

US-CL-CURRENT: 435/6; 204/400, 204/403.15, 422/50, 422/52, 422/55, 422/61, 422/82.05, 422/82.07, 422/82.08, 435/287.1, 435/287.2, 435/288.7, 435/808, 435/810, 435/968, 435/975, 436/164, 436/172, 436/518, 436/524, 436/534, 436/805

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

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☐ 6. Document ID: US 6140045 A

L16: Entry 6 of 15 File: USPT Oct 31, 2000

US-PAT-NO: 6140045
DOCUMENT-IDENTIFIER: US 6140045 A

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**** See image for Certificate of Correction ****

TITLE: Multi-array, multi-specific electrochemiluminescence testing

DATE-ISSUED: October 31, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wohlstadter; Jacob	Cambridge	MA		
Wilbur; James	Rockville	MD		
Sigal; George	Gaithersburg	MD		
Martin; Mark	Rockville	MD		
Guo; Liang-Hong	Laurel	MD		
Fischer; Alan	Cambridge	MA		
Leland; Jon	Silver Spring	MD		

US-CL-CURRENT: 435/6; 204/400, 204/409, 205/777.5, 422/52, 422/58, 422/82.01, 422/82.05, 435/287.1, 435/287.2, 435/288.7 , 435/4, 435/7.1, 435/7.2, 435/808, 435/968, 435/973, 436/172, 436/518, 436/524, 436/525, 436/528, 436/531, 436/805, 436/806

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw. De

☐ 7. Document ID: US 6090545 A

L16: Entry 7 of 15 File: USPT Jul 18, 2000

US-PAT-NO: 6090545
DOCUMENT-IDENTIFIER: US 6090545 A

TITLE: Multi-array, multi-specific electrochemiluminescence testing

DATE-ISSUED: July 18, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wohlstadter; Jacob	Rockville	MD		
Wilbur; James	Rockville	MD		
Sigal; George	Gaithersburg	MD		
Martin; Mark	Rockville	MD		
Guo; Liang-Hong	Laurel	MD		
Fischer; Alan	Cambridge	MA		
Leland; Jon	Silver Spring	MD		

US-CL-CURRENT: 435/6; 204/400, 422/58, 422/61, 422/82.01, 435/287.1, 435/287.2, 435/287.9, 435/4, 435/7.1, 435/7.2, 436/172, 436/518, 436/806

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw. De

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☐ 8. Document ID: US 6066448 A

L16: Entry 8 of 15 File: USPT May 23, 2000

US-PAT-NO: 6066448
DOCUMENT-IDENTIFIER: US 6066448 A

TITLE: Multi-array, multi-specific electrochemiluminescence testing

DATE-ISSUED: May 23, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wohlstadter; Jacob N.	Cambridge	MA		
Wilbur; James	Rockville	MD		
Sigal; George	Gaithersburg	MD		
Martin; Mark	Rockville	MD		
Guo; Liang-Hong	Laurel	MD		
Fischer; Alan	Cambridge	MA		
LeLand; Jon	Silver Spring	MD		

US-CL-CURRENT: 435/6; 204/400, 422/102, 422/58, 422/61, 422/82.01, 435/287.1, 435/287.2, 435/4, 435/7.1, 435/7.2, 436/172, 436/518, 436/806

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWMC | Draw De

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☐ 9. Document ID: US 5883153 A

L16: Entry 9 of 15 File: USPT Mar 16, 1999

US-PAT-NO: 5883153
DOCUMENT-IDENTIFIER: US 5883153 A

TITLE: Fluoride ion sustained release preformed glass ionomer filler and dental compositions containing the same

DATE-ISSUED: March 16, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Roberts; Thomas Arwel	Congleton			GB
Miyai; Kozo	Nara			JP
Ikemura; Kunio	Joyo			JP
Fuchigami; Kiyomi	Kyoto			JP
Kitamura; Toshio	Uji			JP

US-CL-CURRENT: 523/116; 501/151, 523/117, 523/212, 524/443, 524/450, 524/556, 524/845, 524/847, 524/916

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWMC | Draw De

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10. Document ID: US 5858746 A

L16: Entry 10 of 15 File: USPT Jan 12, 1999

US-PAT-NO: 5858746
DOCUMENT-IDENTIFIER: US 5858746 A

TITLE: Gels for encapsulation of biological materials

DATE-ISSUED: January 12, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Hubbell; Jeffrey A.	Austin	TX		
Pathak; Chandrashekhar P.	Waltham	MA		
Sawhney; Amarpreet S.	Newton	MA		
Desai; Neil P.	Los Angeles	CA		
Hill; Jennifer L.	Austin	TX		
Hossainy; Syed F. A.	Austin	TX		

US-CL-CURRENT: 435/177; 424/450, 424/487, 424/497, 424/499, 427/2.14, 427/2.21, 427/213.3, 427/213.32, 427/213.34, 427/213.36, 514/2, 514/772.1, 514/773, 514/777, 524/56, 524/702, 524/704, 524/733, 524/734, 524/849, 524/850, 524/852, 524/856, 525/408, 525/413, 525/54.1, 525/54.2, 528/361

Terms	Documents
L15 and acryloyl	15

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